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ESTIMATED DATA ON THE ENERGY, GASEOUS, AND WATER METABOLISM OF POULTRY FOR USE IN PLANNING THE VENTILATION OF POULTRY HOUSES

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INTRODUCTION

There seems to be a dearth of reliable estimates of the production of heat, carbon dioxide, and water by the different classes of poultry, for the use of agricultural engineers in the designing of ventilating systems for poultry houses. This paper presents a series of estimates of the nature required, covering the entire growing period of a large (Plymouth Rock) and a small (Leghorn) breed of chickens, of the Bronze turkey, of the Pekin duck, and of Embden and Toulouse geese.

In the Divisions of Animal Nutrition and Poultry Husbandry of the University of Illinois, an intensive study of the growth, metabolism, and nutritive requirements of chickens has been under way for several years, and a similar project on the Bronze turkey has been initiated. The information thus collected and in part published (*22, 23, 24, 25, 26, 27*) [2] was used as the basis of most of the estimates herein reported. Direct application of this information has been made to the two breeds of chickens included in the series of estimations made, and indirect application has been made to turkeys, ducks, and geese, on the basis of a number of assumptions, very few of which can be defended except by the logic of analogy. The excuse for proceeding on this basis is (*24*) that, on the few specific phases of metabolism for which information on a number of kinds of poultry is available, no distinct differences have been noted, and, moreover, (*25*) no other basis of estimation appeared at hand.

METHODS OF ESTIMATION

The general procedure followed in making the estimations has been to factor out and to assess as accurately as possible all of the items contributing to the energy requirements and expenditures of the birds and to their requirements for water, and then to integrate these factors for birds of different weights, to get the values desired. In pursuing this course a number of assumptions have been made. These, as well as the integrations leading to the information desired, are here described.

The basal metabolism of all classes of poultry per square meter of body surface is assumed to be the same at equivalent ages, and to be equal to the values reported by Mitchell, Card, and Haines (*23*) in a comprehensive investigation of the metabolism of chickens.

[1] Received for publication June 14, 1933; issued January, 1934.

[2] Reference is made by number (italic) to Literature Cited, p. 747.

The basal metabolism of mature chickens averages about 800 cals. per square meter for males and 750 cals. per square meter for females. Herzog (*13*) has reported an average of 874 cals. for six pigeons, and values of 907, 891, and 1,093 cals. for a gander, a goose, and a duck, respectively. These values, and especially the last, probably include some heating effect of food, since the pigeons were fasted only for 20 to 24 hours, the geese for 12 to 16 hours, and the duck for 8 to 12 hours. Workers at this laboratory have found that in determining basal metabolism a fasting period of 48 hours is required for chickens in order that there may be no residual effect of the last feeding; Benedict and Riddle (*3*) found that for pigeons a fast of 24 hours is required for the attainment of fasting respiratory quotients. Riddle, Nussmann, and Benedict (*30*) measured the basal metabolism of the common pigeon from an age of 21 days to an age of 439 days, and observed essentially the same relations as those obtained for chickens. Finally, Hári obtained measurements of 682 to 1,038 cals. per square meter for the basal metabolism of geese (*10*) and 735 to 935 cals. for ducks (*11*).

These citations justify the essential accuracy of the assumption that the basal metabolism of all poultry is much the same at equivalent ages, i.e., ages at which the same fraction of the mature weight has been attained. The disagreement between the values cited, aside from technical errors, may be largely due to the use of formulas for estimating surface area from weight which possess different degrees of accuracy.

The extra heat due to voluntary activity is assumed to approximate one half the basal heat. The justification of this relation for chickens is discussed fully by Mitchell, Card, and Hamilton (*25, p. 133*). The absence of any information whatever on this point for other classes of poultry necessitated the general use of this figure throughout. Specific investigation with turkeys, ducks, and geese will be required before the magnitude of the error incurred by proceeding on this assumption can be determined.

Although specific information was found with reference to the growth rates of the different classes of poultry, the energy content of the gains had to be computed from the data available for chickens. The data for White Plymouth Rocks were taken directly from Illinois Bulletin 276 (*24*), and those for White Leghorns from Illinois Bulletin 367 (*25*).

The chemical composition of turkeys, ducks, and geese has been but little studied. From such analyses as were available, however, (*1, 12, 18, 19*) it appears that turkeys contain about as much fat as chickens, ducks are considerably fatter, and geese even more so. In making the computations summarized in the tables it was assumed that, at equivalent ages, turkeys put on gains in weight possessing the same chemical composition as the gains of chickens, but that ducks put on gains containing half again as much fat, and geese put on gains containing twice as much fat.

The total net energy requirement is, of course, equal to the sum of the energy expended in basal metabolism, the energy expended in muscular activity, and the energy equivalent of the gain in weight.

The net energy requirement is stated also in terms of dry matter required, the dry matter being assumed to have the net energy value of wheat for chickens (*18, 19*), i.e., 282 cals. per 100 g.

The heating effect of the feed is taken at 68 cals.[3] per 100 g of dry matter, this being the heat increment of wheat for chickens (*18*, *19*).

The total heat production is the sum of the basal heat, plus the heat liberated in muscular activity, plus the heat increment due to feed.

In estimating the output of carbon dioxide from the total heat production, an average respiratory quotient of 1 was assumed for the day. The justification for this assumption is found in the respiration experiments of Mitchell and Haines (*27*) on chickens and the measurements on their control birds by Baldwin, Nelson, and McDonald (*2*) and by Caridroit (*5*). On this assumption, 1 l of CO_2 is produced for each 5.047 cals. of heat.

The estimations of water metabolism involved all of the following factors: (*a*) Water-vapor elimination, (*b*) excretion of liquid water in the excrement, (*c*) production of metabolic water in the body, and (*d*) storage of water in the tissues during growth. Having estimated these factors according to what seemed to be reasonable procedures, the water requirement was computed as being equal to $a+b+d-c$.

From Hâri's respiration experiments on geese (*10*), it appears that at 16° C. an average of 20 percent of the heat output is lost in the vaporization of water, and at 28° an average of 40 percent is thus lost. At a body temperature of approximately 41° all the heat produced must be lost as vaporized water. These meager figures on geese, as well as data on other animals that might be cited, indicate clearly a rapidly increasing percentage of heat lost as vaporized water as the environmental temperature increases. This relation appears to be a logarithmic one, and Hâri's data are well described by the following equation, the constants of which were determined by the writers.

$$W=7.14\ e^{0.0643t}$$

in which W is the percentage of the heat produced that is dissipated as the heat of vaporization of water, and t is the environmental temperature expressed in degrees centigrade. From this equation the following values were computed:

°C	°F.	W
0	(32)	7. 1
4. 5	(40)	9. 5
10	(50)	13. 6
15. 5	(60)	19. 4
21. 0	(70)	27. 6
26. 5	(80)	39. 3
32. 2	(90)	56. 7
37. 7	(100)	81. 1
41	(106)	100

In the estimations made on all classes of poultry it was assumed that 40 percent of the heat production was represented in the vaporized water and that this condition, in normally feathered birds, obtained at 28° C. (82° F.). Hence the vaporized water at this temperature was estimated from the total heat production by multiplying by 0.4 and dividing by 0.58, the heat of vaporization of 1 g of water at 30° C. (86° F.), a figure approximating average skin

[3] No allowance for a variable heating effect of feed as the level of feeding is varied can be made in the absence of information on this point. However, it has been found (*27*) that the heating effects of 50 and 75 g of corn for chickens are much the same per 100 g of corn fed.

temperature. For other environmental temperatures, the vaporized water may be computed by means of the tabulation given above. The maximum vaporized water, obtained at temperatures equal to or exceeding body temperature is, of course, computed from the total heat production merely by dividing by 0.58. It should be pointed out here that heat lost in vaporized water is not available for heating the surroundings unless the water vaporized is condensed.

The elimination of liquid water in the excreta was computed from the dry matter consumed on the assumption that the dry matter was 20 percent indigestible and that the mixed excreta contained 27 percent of dry matter. The latter value was obtained from a number of published digestion experiments on chickens in which the mixed excreta were analyzed for moisture. It is recognized, of course, that this value is subject to variation.

The stored water was computed from the daily gains in each species and the composition of gains in chickens as determined at the Illinois Station.

The metabolic water, that is, the water produced in the body by the oxidation of the food nutrients, was computed on the basis of the following commonly used factors:

100 g of protein yield 41 g of water.
100 g of fat yield 107 g of water.
100 g of carbohydrate yield 60 g of water.

Considering the fact (1) that the metabolic mixture from which the metabolic water originates is equal to the mixture of nutrients consumed minus the mixture of nutrients stored in the body, and (2) that the nutrient mixture in wheat, in terms of which the dry-matter requirements are expressed, is mainly carbohydrate in character, it was decided to compute the metabolic water in all cases as 48 percent of the dry-matter requirement. It is realized that the metabolic water might have been estimated by a more refined method that would have taken into account the varying rates of storage of nutrients at different stages of growth, but the greater accuracy thus obtained would have been secured at the expense of much greater labor, while the error incurred by the simpler method is a relatively small item in the water values desired, and is merely one of a large number of errors to which the estimation of each of the factors in the problem are undoubtedly subject.

For Bronze turkeys, the growth data of Funk and Margolf (*8*) have been used. The growth obtained in these experiments is considerably more rapid than that reported by Mussehl (*28*). In fact, it does not show the slowing that might have been expected at the greater ages, and no success was had in fitting to the data the mathematical expression of the law of diminishing returns that has been used so successfully by Brody in describing the growth of animals for all but the first small fraction of the growth period. The adult weight of the male Bronze turkey is taken to be 36 pounds and that of the female 20 pounds.

For Pekin ducks, the data of Horton and the mathematical analysis of them made by Titus (*36*) have been followed. These data were obtained on a flock in which the sexes had not been separated. The growth rate in this flock was somewhat greater than that later reported by Horton (*15*) and also than that reported by Lehmann (*21*), by

Spyra (*33*), or by Tallent (*34*). Some unpublished data obtained through the courtesy of Professor Carrick, of Purdue University, show that more rapid growth of Pekin ducks is possible for the early weeks of life, but these data show a much less sustained growth than those of Horton. The adult weight of the male Pekin duck is said to be 9 pounds and that of the female 8 pounds. The large majority of all ducks raised in this country are of the Pekin breed.

The only comprehensive study of the growth of geese that has been found in the literature is that of Cushman (*6, pp. 327–358*) obtained at the Rhode Island Agricultural Experiment Station and published in 1895. Since the Embden and Toulouse are the breeds of geese most commonly raised in this country and also the breeds used to the greatest extent in the Rhode Island experiments, no other breeds are considered in this paper. Although the adult weights of Toulouse geese are greater than those of Embden, i.e., for the males, 26 pounds as compared with 20 pounds, and for the females, 20 pounds as compared with 18 pounds, the rates of growth as observed by Cushman are quite similar up to a body weight of 11 pounds. Hence the growth of both breeds has been calculated by the same equations obtained from the combined data of Cushman. Up to a weight of 2 pounds the growth is well described by the equation

$$W = 98.63\ e^{0.0718t}$$

and from this weight to a weight of 11 pounds by the equation

$$W = 9000 - 9609\ e^{-0.0545t}.$$

In both of these equations W is the body weight in grams at age t, expressed in weeks from hatching. That an error is incurred in extending this equation to both breeds beyond a body weight of 11 pounds is, of course, apparent. However, neither for geese nor for turkeys and ducks are the estimates at adult weights affected in the slightest by any errors inherent in the method used for the estimation of growth or of growth requirements.

In determining the basal metabolism of birds of given weights, the surface areas were computed by the use of the following formulas:

Chickens:

$$S_{\text{sq. cms.}} = 8.19\ W^{0.705}_{\text{gms}}$$

Turkeys:

$$S_{\text{sq. cms.}} = 13.16\ W^{0.667}_{\text{gms}}$$

Ducks:

$$S_{\text{sq. cms.}} = 9.02\ W^{0.705}_{\text{gms}}$$

Geese:

$$S_{\text{sq. cms.}} = 10.45\ W^{0.667}_{\text{gms.}}$$

The formula for chickens was obtained for the Leghorn breed (*22*); that for turkeys was obtained from unpublished data on the surface area of Bronze turkeys as determined by the mold method; that for ducks represents a more or less arbitrary raising of the constant in the formula for chickens in accordance with the higher Meeh constant commonly used for ducks and geese; and that for geese is the Voit formula for this species.

DISCUSSION OF ESTIMATED DATA

The final computations are given in tables 1 and 2. It will be noted that although the ages corresponding to the given weights are estimated for the different sexes (except for ducks and geese, for which

the data are not available), no distinction between the sexes is made with respect to the other values in the tables. For chickens and turkeys the computations were carried out in full for the two sexes, but it was then observed that the values for the different sexes at any given weight did not differ greatly, and in the large majority of cases were very nearly identical. This is because no considerable sex differences had been assumed throughout the calculations except with respect to the rates of growth and the composition of gains in weight. The faster rate of growth of the male is almost exactly compensated for, so far as the energy requirements for growth are concerned, by the higher fat content of the gains in weight put on by the female.

TABLE 1.—*Daily food requirements, heat production, and water used and excreted by chickens and turkeys of different weights*

Body weight (pounds)	Age		Total net energy required	Dry matter required [a]	Total heat produced	CO_2 produced	Water required at 28° C.	Water vaporized at 28° C.	Total water excreted at 28° C.	Maximum water vaporized at 41° C.
	Males	Females								
	Weeks	*Weeks*	*Calories*	*Grams*	*Calories*	*Liters*	*Grams*	*Grams*	*Grams*	*Grams*
WHITE LEGHORN CHICKENS										
0.077	0	0	13	5	10	2	14	7	11	17
0.5	4.0	4.4	80	28	82	16	72	56	77	141
1.0	6.9	8.2	104	37	110	22	99	76	103	190
1.5	9.6	11.5	124	44	132	26	117	91	124	228
2	12.5	14.8	143	51	155	31	134	107	144	267
3	18.2	22.4	173	61	194	38	161	134	179	334
4	25.4	38.3	204	72	235	47	188	162	216	405
5 [b]	33.0	.....	235	83	281	56	223	194	255	484
WHITE PLYMOUTH ROCK CHICKENS										
0.077	0	0	13	5	10	2	14	7	11	17
0.5	4.6	4.6	79	28	82	16	69	56	77	141
1	6.6	6.7	108	38	110	22	96	76	104	190
1.5	8.7	8.9	137	49	135	27	118	93	128	233
2	9.7	10.7	160	57	159	32	137	110	152	276
3	12.4	14.9	201	71	200	40	168	138	191	345
4	15.7	19.4	233	82	238	47	195	164	224	410
5	19.3	25.4	258	92	280	55	224	193	261	482
6	23.3	.....	259	92	310	62	241	214	282	535
7 [b]	.....	.....	285	101	354	70	270	244	319	610
BRONZE TURKEYS										
0.115	0	0	24	8.5	20	4	22	14	20	34
0.5	3	3+	95	34	115	23	98	79	104	198
1	5	5+	150	53	170	34	146	117	156	293
2	7	8	221	79	239	48	208	165	223	412
3	9	10−	275	98	287	57	265	198	271	495
4	10+	11	322	114	327	65	304	226	310	564
5	11+	12+	364	129	365	72	337	252	347	629
7	13	15	441	157	437	87	391	301	417	754
10	16	19	553	196	553	110	480	381	526	954
15	20	.....	706	251	720	143	607	496	682	1,241
20	25	.....	840	298	890	176	743	614	835	1,534
20	.....	52	645	229	801	159	611	552	721	1,381
36	104	.....	1,017	361	1,262	250	963	870	1,137	2,176

[a] These values relate to dry matter with the net energy value of wheat, i.e., 282 cals. per 100 g.
[b] Male.

TABLE 2.—*Daily food requirements, heat production, and water used and excreted by ducks and geese of different weights*

PEKIN DUCKS

Body weight (pounds)	Age	Total net energy required	Dry matter required [a]	Total heat produced	CO_2 produced	Water required at 28° C.	Water vaporized at 28° C.	Total water excreted at 28° C.	Maximum water vaporized at 41° C.
	Weeks	*Calories*	*Grams*	*Calories*	*Liters*	*Grams*	*Grams*	*Grams*	*Grams*
0.125	0	23	8	18	4	18	12	18	31
0.5	2.0	110	39	97	19	96	67	96	167
1	3.2	158	56	135	27	128	93	134	233
1.5	4.1	205	73	166	33	159	114	168	286
2	4.8	255	90	196	39	187	135	202	338
3	6.2	328	116	252	50	232	174	260	434
4	7.5	382	135	300	59	266	207	307	517
5	8.9	401	142	342	68	290	236	341	590
6	11.1	402	143	375	74	307	259	365	647
7	13.8	394	140	404	80	319	279	383	697
8 [b]		328	116	407	81	311	281	367	702
9 [c]		381	135	473	94	361	326	426	816

TOULOUSE AND EMBDEN GEESE

0.215	0	31	11	28	6	26	19	27	48
0.5	1.7	115	41	113	22	81	78	86	195
1	3.1	179	63	154	30	145	106	153	266
1.5	3.8	233	83	182	36	185	126	192	314
2	4.4	267	95	205	41	214	141	218	353
3	4.6	313	111	242	48	244	167	258	417
4	5.8	343	122	270	53	254	186	275	466
5	6.9	370	131	308	61	273	212	299	531
6	8.2	390	138	339	67	296	234	329	584
8	11.1	430	152	392	78	332	270	380	676
10	14.5	463	164	446	88	370	308	429	769
12	18.7	488	173	493	98	400	340	468	850
15	27.5	514	182	561	111	444	387	522	967
18	45.4	526	187	617	122	474	426	561	1,064
20		529	188	654	130	496	451	586	1,128
26 [c]		649	230	805	160	615	555	725	1,388

[a] These values relate to dry matter with the net energy value of wheat, i.e., 282 cals. per 100 g.
[b] Female.
[c] Male.

It is important to check such calculations as those given in tables 1 and 2, insofar as possible, from published information on the food intake or water intake, for example, of poultry on feeding experiments. When such comparisons are made of the dry-matter requirements computed for birds of definite weights and the actual amounts of feed consumed by birds of equal or similar weight in actual experiments, the latter values will in all cases be the larger for a number of reasons. In feeding experiments there is an inevitable scattering and wastage of food which may amount to as much as 5 or 10 percent. The food weighed out to the birds is not moisture-free, but probably contains as much as 10 to 15 percent of water. Mixed poultry rations may have a lower net energy value than wheat. When proper consideration is given to these points of difference, the values for the estimated dry-matter requirements of chickens, turkeys, and ducks do not seem to be unreasonably low, particularly when it is remembered that they have been computed on the assumption of a degree of activity which may be too low for birds not closely confined. Such comparisons may be made from the food-intake figures for chickens given by Jull (*16*) and Jull and Titus (*17*), those for turkeys given by Funk and Margolf (*8*), and those for ducks given by Titus (*36*) and Lehmann (*21*). In Lehmann's experiments the dry-matter intakes

are given for ducks at different stages of growth. Since this affords the most exact comparison of any available, the average values in these experiments for Lehmann's groups IA, IB, and IC, totaling 84 birds, are given (table 3).

TABLE 3.—*Dry-matter intake of ducks at different stages of growth in Lehmann's experiments*

Age in weeks	Average body weight	Dry matter consumed daily	Age in weeks	Average body weight	Dry matter consumed daily
	Grams	*Grams*		*Grams*	*Grams*
0	125		5	1,434	144
1	260	32	6	1,686	153
2	539	74	7	1,897	157
3	932	120	8	1,967	158
4	1,207	125			

These ducks, growing at a somewhat more rapid rate than that assumed in the construction of table 2, consumed dry matter in very nearly the same amounts as the estimated requirements.

No published information could be found on the water requirements of poultry and only one reference to observed water consumption (7), which related to mature White Leghorn hens while laying eggs. However, through the kindness of Dr. L. E. Card and Dr. H. J. Sloan, of the Division of Poultry Husbandry, records were obtained of the food and water consumption of a number of pens of young chicks on different rations, as well as of laying hens. The significant value in this connection was considered to be the ratio of the weight of water drunk to the weight of dry food consumed. For 10 pens of chicks the average ratio for the first 10 to 12 weeks of life was 2.17 g of water to 1 g of feed. If it be assumed that the wastage of water was no greater than the wastage of feed, and that the feed contained 10 percent of moisture, then the average ratio of water to dry matter would be 2.52 to 1.

In one pen of Rhode Island Red hens (laying) containing 45 to 47 birds per pen, the ratios of water to feed consumed in 4 successive weeks were 1.70, 1.83, 1.42, and 1.42, the average being 1.59. In one pen of White Leghorn hens, containing 41 to 45 birds per pen, the ratios for successive weeks were 2.01, 1.76, 1.41, and 1.35, the average being 1.62. On the same assumptions as before, the average ratio of water to dry matter consumed becomes 1.89 to 1. The difference between the two sets of data—i.e., those for chicks and laying hens—may have been due in part to the fact that the former were collected mainly in the warm months of June, July, and August, while the latter were collected from April 7 to May 4, inclusive.

If the ratios of the estimated amounts of water to dry matter required are computed from the data in tables 1 and 2, they will be found to vary from 2.0 to 1 to 2.7 to 1 and to average close to 2.4 to 1. These results are in good agreement with the chick data cited above, and apply to fairly warm weather. For growing mice, Bing and Mendel (4) have reported an average ratio of 1.3 g of water consumed to 1 g of food. The senior writer has obtained values for poultry approximating this one, if the water requirement is assumed to equal 800 cc per square meter of body surface, as Richter and

Brailey (*29*) have found with growing rats. However, the weight of evidence seems to establish the fact that the water requirements of poultry are higher than these estimates indicate, possibly because of the higher body temperature.

In using the water data in tables 1 and 2, it should always be remembered that these data are subject to the greatest variation of all. They are dependent particularly upon the environmental temperature, as pointed out above, and also, in an undetermined manner, upon the environmental humidity, a high humidity tending to depress the vaporization of water from the body. Probably also changes in the feather covering have a considerable effect on the vaporization of water, and the character of the ration fed affects the amount of water consumed. The content of the diet in protein and in salts that are excreted by the kidney must also modify the amount of water consumed, the relation being a direct one.

If the environmental temperature sinks to very low levels, the heat production of poultry will increase beyond the values given in the tables, in order to maintain normal body temperatures. The increase will be secured either by an increased muscular activity or by an increased muscle tonus, or by both; or possibly even by an increased consumption of feed. Some rough estimates may be made of the environmental temperature at which an increase in heat production must occur. For these calculations a critical temperature for the fasting bird of 62° will be used. This temperature was determined by Mitchell and Haines (*26*) in experiments with a considerable number of chickens. Terroine and Trautmann (*35*), however, have reported higher critical temperatures for a hen and a goose. For a bird consuming food and moving about normally, there is an excess production of heat above the basal which, at temperatures below 62° is available to maintain body temperature. This excess heat consists of (1) the heat liberated by muscular activity, assumed in these calculations to equal one half of the basal heat, and (2) the heat increment produced by the feed, assumed to equal 68 cals. per 100 g of dry matter consumed. For the different classes of poultry and for the different body weights, the extra heat available for this purpose is given in table 4. Table 4 gives for each species an estimate of the heat required to maintain body temperature, in excess of that furnished by the basal metabolism, for each degree of fall in environmental temperature below 62°. This estimate is based on the validity of Newton's law of cooling bodies as applied to the bird under basal conditions at its critical temperature (*26, p. 556*), and is equal to the basal heat production of the bird divided by 44, the difference between the body temperature, 106°, and the critical environmental temperature in the fasting condition, 62°. Table 4 also gives for each species an estimate of the critical temperature of the active bird consuming its required food.[4] This temperature would be considerably higher for the bird while asleep or otherwise inactive.

[4] For instance, for a 2-pound Pekin duck the critical temperature = 62 − (106 ÷ 2.0) = 9.

TABLE 4.—*Calculation of critical temperatures for different species of poultry of varying weights*

Body weight (pounds)	White Leghorn chickens			White Plymouth Rock chickens			Bronze turkeys			Pekin ducks			Geese		
	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temperature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temperature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temperature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temperature	Extra heat available	Heat required per ° F. drop below 62° F.	Critical temperature
	Cals.	*Cals.*	*° F.*	*Cals.*	*Cals.*	*° F.*	*Cals.*	*Cals.*	*° F.*	*Cals.*	*Cals.*	*° F.*	*Cals.*	*Cals.*	*° F.*
0.5	42	1.02	21	42	1.02	21	46	1.11	21	52	1.14	16	44	.89	13
1	51	1.2	19	52	1.2	19	86	2.1	21	68	1.4	13	80	1.7	15
1.5	64	1.5	19	67	1.5	17				88	1.7	10	101	2.0	12
2	75	1.8	20	79	1.8	18	115	2.8	21	106	2.0	9	117	2.2	9
3	94	2.3	21	99	2.3	19				136	2.6	10	138	2.7	11
4	111	2.8	22	118	2.8	20	158	3.6	18	161	3.2	12	142	2.8	11
5	131	3.4	23	135	3.4	22	175	4.0	18	179	3.7	14	154	3.2	14
6				146	3.8	24				190	4.2	17	171	3.6	14
7				164	4.3	24	216	5.0	19	203	4.7	19			
8										188	5.0	24	198	4.3	16
9										219	5.8	24			
10							274	6.4	19				223	5.0	17
12													242	5.7	20
15							353	8.3	19				269	6.6	21
18													290	7.5	23
20							402	10.1	22				303	8.0	24
26													372	9.8	24
36							584	15.4	24						

All such estimates as those above given are subject to great variation. The critical temperature will be higher (1) the less fat the bird contains, (2) the less active it is, (3) the less complete its coat of feathers, (4) the more humid its surroundings, (5) the less food it consumes and the longer the time from the peak of its digestion, (6) the larger the volume and the lower the temperature of the water drunk, and so forth.

No estimates have been made for the female bird while laying eggs. Under this condition, the net energy requirements are increased, as are also the consumption of dry food and water and the elimination of heat, carbon dioxide, and moisture. Thus, if a pullet is producing one egg a day of average size (58 g), its net energy requirements will be increased by 95 cals. (*25*), its dry-matter (wheat) requirements by 34 g ($95 \div 2.82$), its heat production by 23 cals. (0.34×68), and its carbon dioxide production by 4.5 l ($23 \div 5.047$), etc. The water requirements will be increased in proportion to the increase in dry feed ($34 \text{ g} \times 2.2 = 75 \text{ g}$) and to the water stored in the egg ($58 \text{ g} \times 0.75 = 43 \text{ g}$), making a total increase of 118 g. Estimates for ducks and geese may be computed from the analyses of duck and goose eggs made by Hepburn and Katz (*12*). These estimates are given in table 5. The average egg production of a duck, according to Lamon and Slocum (*20*), ranges from 80 to 120 per year; that of a goose ranges from 12 to 36.

TABLE 5.—*Chemical composition of duck and goose eggs*

Kind of egg	Average weight	Shell	Chemical composition of edible portion			Total constituents in average egg			
			Water	Fat	Protein	Water	Fat	Protein	Energy
	Grams	*Percent*	*Percent*	*Percent*	*Percent*	*Grams*	*Grams*	*Grams*	*Calories*
Duck [a]	91	10.6	68.5	14.4	13.6	56	11.7	11.1	174
Goose [b]	205	12.7	71.2	12.2	14.1	127	21.8	25.2	351

[a] Taken from Horton (*15a*).

[b] Taken from Lamon and Slocum (*20*).

DISCUSSION OF THE DATA FROM AN ENGINEERING STANDPOINT

The agricultural engineer has long needed some source to which he might turn for such essential metabolism data relating to poultry as could be used in designing heating or ventilating systems for poultry houses. The data here recorded give the basal and normal heat production of various breeds of chickens, ducks, geese, and turkeys from the time of hatching to approximate maturity. Hence the data are applicable under a wide range of conditions. A single example of their application may be of value.

Assume an ordinary shed-roof house, 20 by 30 feet, housing 200 White Plymouth Rock hens averaging 4 pounds in weight. The floor area is 600 square feet and the volume approximately 4,200 cubic feet. Referring to table 1 we find that a 4-pound White Plymouth Rock bird will consume 82 g of dry matter, produce 238 calories of heat and 47 liters of carbon dioxide, and vaporize 164 g of water daily. If these data are converted into terms commonly used by engineers, 200 hens will produce hourly 7,866 B.t.u. and vaporize 3 pounds of water per hour, making 21,000 grains of moisture to be removed by ventilation. However, these data are given for an environmental temperature of 82° F. At this temperature an ordinary poultry house would be wide open. If a house temperature of 50° F. is assumed,[5] the moisture production would be much less. The tabulation given on page 737 shows that at this temperature 14 percent of the heat produced is dissipated as water vapor, which, according to the method of estimation herein reported, would make 57 g of water produced daily by such a bird. $((238 \times 0.14) \div 0.58 = 57$; $57 \times 200 = 11{,}400$ g daily, or 7,315 grains or 1.045 pounds hourly.) This represents the amount of water to be removed hourly from the henhouse.

The temperature maintained within the house depends upon the balance between the heat produced and the heat saved. The data given in tables 1 and 2 are for temperatures of 28° and 41° C. (82.4° and 105.8° F.).

Tests in Wyoming (*14, pp. 13–16*) showed that in moist air the combs froze at 6° F.; at 8° to 10° egg production was reduced 25 percent; and at 0° the hens stopped laying. Other tests reveal that in an uninsulated house a temperature of 7° to 10° higher than that outside may be obtained, whereas in an insulated and ventilated house the difference may be as great as 16° to 30°.

Ruckman (*31*) found, in a large poultry feeding plant, that at a temperature below 60° F. the consumption of feed per pound of gain increased; and that at 65°, although the birds were healthy, they showed a tendency to lose their appetites and failed to gain. He also found that too high humidity made breathing difficult and too low humidity produced a dry skin and a poor growth of feathers.

The ventilation problem requires a consideration of carbon dioxide and moisture production and removal. Many writers state that it is desirable to maintain a purity of 9 parts of CO_2 to 10,000 parts of air. Purity of air is always to be desired, if it can be obtained without harmful drafts. A higher percentage of CO_2 than that mentioned above may be maintained in the henhouse without apparent harm, but too high a concentration of CO_2 in the incubator room may mean a decreased hatch. At the Iowa Agricultural Experiment

[5] This is still above the estimated critical temperature of a 4-pound White Plymouth Rock bird, which is 20° F. (table 4).

Station (*9*) it was found that 11 parts to 10,000 could be obtained with a circulation of 4.2 cubic feet of air per hour per bird, which in this case was approximately 0.3 dilution of air per hour.[6]

There is some doubt as to the amount of water that can be removed from the litter by ventilation. Much depends upon the floor temperature, the vapor pressure balance of the air entrapped in the litter and that just above, and the depth and kind of litter.

The quantity of air required for the removal of the average production of moisture will vary with variations in the air temperature and the relative humidity. Since it has been shown that 4.2 cubic feet of air per hour are required to maintain what may be considered a desirable limit of CO_2, we can now ascertain the amount of air necessary to remove the moisture at an assumed temperature of 50° F. and 75 percent relative humidity within the henhouse and 15° and 80 percent humidity of the admitted air. Air at 50° with a relative humidity of 75 percent holds 3.057 grains of moisture per cubic foot and the outside entering air would bring in 0.789 grain. Hence to remove 7,315 grains per hour would require $7,315 \div (3.057 - 0.789) = 3,225$ cubic feet per hour, or 16 cubic feet per hour per bird. One British thermal unit will heat 53 cubic feet of air 1°. Hence it will require 2,128 B.t.u. per hour to heat 3,225 cubic feet of incoming air to the temperature of the henhouse.

A 4-pound White Plymouth Rock bird produces 238 calories of heat daily. Two hundred such birds would therefore produce 7,866 B.t.u., hourly. However, only 6,765 B.t.u. are dissipated as sensible heat, the remaining 14 percent being lost in the vaporization of water. The air necessary to remove the moisture produced requires 2,128 B.t.u., leaving a remainder of 4,637 B.t.u. to take care of the heat losses from the house.

The above-mentioned house has an equivalent surface area in walls and roof of 1,336 square feet and 50 square feet of glass. If the walls are insulated to provide a loss of only 0.22 B.t.u. per square foot per degree of difference in temperature per hour, there would be a total loss of 294 B.t.u. from the wall plus 55 B.t.u. from the single glass window, or a total of 349 B.t.u. per hour per degree of difference in temperature.

Thus the heat available would permit the maintenance of 13.3° difference in temperature, whereas under the foregoing assumption a difference of 35° was desired. If the insulation value were increased and storm windows used, a greater temperature difference would be obtained. Moreover, if larger quantities of feed were consumed more heat would be produced, but it might be more economical to provide artificial heat in order to maintain the desired temperature. It should also be noted that no allowance of feed energy for egg production has been made.[7]

The foregoing example shows that it is impractical to maintain a temperature of 50° in such weather and provide the amount of ventilation mentioned without the use of artificial heat. If the results of the Iowa tests are accepted, less ventilation can be used without producing harmful concentrations of CO_2. However, under

[6] On the other hand, in respiration experiments animals may be maintained in apparently normal condition in chambers containing as high as 0.5 to 1 part of CO_2 per 100. In the laboratory of the senior author experiments with poultry have been carried out for many days in succession with such concentrations of CO_2 prevailing.

[7] If the 200 hens were producing 100 eggs daily, their heat production will be increased 2,300 cals. daily or about 342 B.t.u. hourly. This extra heat would permit another degree difference in temperature between henhouse and exterior.

the temperature conditions assumed above, less ventilation would hardly be adequate for moisture removal. The removal of moisture is a major problem in poultry houses. Poultry have no sweat glands, but they give off relatively large amounts of vapor in respiration and through the skin.

It was found at the Nebraska station (*32*) that maximum egg production was obtained when temperatures were not permitted to fluctuate widely. A henhouse temperature of 50° F. is too high to be maintained on most farms in winter without artificial heat. Hence a lower temperature held uniformly would be desirable. Increasing numbers of poultrymen have had success with artificial heat properly regulated, but failure has commonly resulted when temperatures were allowed to go too high or to fluctuate widely.

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STEWART'S WILT DISEASE OF CORN, WITH EMPHASIS ON THE LIFE HISTORY OF PHYTOMONAS STEWARTI IN RELATION TO PATHOGENESIS[1]

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INTRODUCTION

The studies reported in this paper were made on certain phases of Stewart's wilt disease of corn, caused by *Phytomonas stewarti* (Smith) Com.S.A.B., particular emphasis being placed on the life history of the causal organism in relation to pathogenesis. The difficulties encountered in studying certain stages of the life history of this organism and in making isolations from badly contaminated material and from the soil, made the development of a selective medium highly desirable. Such a medium was developed through studies of the physiology of the causal bacteria. Other bacterial forms causing symptoms similar to those produced by the Stewart's wilt organism are considered briefly. Likewise, some symptoms produced by the wilt organism, not hitherto described or only partly described, are reported.

Four different phases in the life history of the pathogene in relation to pathogenesis are considered: (1) Its entrance into the host plant, (2) its location in the tissue of the host, (3) its exit from the host, and (4) its mode of transmission to a new host.

EARLIER WORK

The first published report of Stewart's wilt disease of corn appeared in 1897 (*16*).[3] The only known host of the wilt-disease organism is the corn plant, *Zea mays* L. It attacks several kinds of corn but its preferred host is sweet corn. Studies on varietal susceptibility have been carried out by several workers, including Reddy (*10*), Rand and Cash (*8*), Reddy and Holbert (*11*), and Holbert, Elliott, and Koehler (*4*).

The wilt disease is rather widely distributed in the United States. It is found throughout the central and eastern sections and recently has been reported from the Pacific coast. So far as the writer is aware, this disease is not known to occur outside of continental United States and Puerto Rico. Its economic importance as evidenced by the losses which it causes is great at times. Occasionally an entire corn crop has been ruined, and losses of from 20 to 40 percent have been frequent (*16*). Unpublished reports estimate the losses in the summer of 1931 and 1932 at certain localities as high as 90 percent of the entire crop of the Golden Bantam variety.

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[3] Reference is made by number (italic) to Literature Cited, p. 769.

Certain symptoms of the disease have been described by Stewart (*16*) and Smith (*15*). They found that the leaves of diseased plants wither gradually. The stem shows no external symptoms, but when cut lengthwise the fibrovascular bundles appear as yellow streaks in the white parenchyma. If the stem is cut crosswise, a yellow viscid bacterial exudate soon oozes from the vascular bundles. This is considered by Stewart and Smith the most characteristic symptom of the disease. The male inflorescence develops prematurely and becomes dry. The whole plant is stunted and bears imperfect, small ears.

The causal organism of this disease was described by Smith as *Pseudomonas stewarti* (*12*). Later it was renamed *Aplanobacter stewarti* (E. F. Smith) McCulloch (*6*). According to Bergey the name is *Phytomonas stewarti*.

The physiology of *Phytomonas stewarti* has been studied by Smith (*13*, *15*). The work on its life history in relation to pathogenesis has been done mainly by Smith (*14*, *15*), Rand (*7*), and Rand and Cash (*8*, *9*). The bacteria enter the host plant, according to Smith (*15*), chiefly through the water pores of young seedlings. The exit of the bacteria from the host plant has been reported as taking place in the form of exudate from the stomata of the husks (*15*, *16*). The transmission of the bacteria to a new host was found by Smith (*14*) to take place through infected seed and by Rand (*7*), and Rand and Cash (*8*, *9*), through the agency of aboveground insects.

The soil relations of the causal organism have received but slight attention. So far no mention has been found in the literature of the isolation of the bacteria from the soil.

MATERIALS AND METHODS

The culture of *Phytomonas stewarti* used in this investigation was isolated from a diseased sweet-corn plant obtained from Dr. A. G. Johnson, Washington, D.C. This culture was purified by several platings and single-cell isolation and its identity established by studies of its physiology and pathogenicity.

Several varieties of maize were used in this work. The sweet-corn varieties included Golden Bantam, which is particularly susceptible to Stewart's wilt disease, Golden Sweet, and Country Gentleman. Two hybrids of sweet corn, known as "hybrids 66 and 70", were also used. Hybrid 66 had been found by G. M. Smith, from whom it was obtained, to be more or less resistant to the disease, and hybrid 70 very susceptible. The field-corn varieties used in some of the trials were Golden Glow, Leaming, and an unnamed variety of dent corn. Unless otherwise stated, Golden Bantam sweet corn was used throughout the trials.

Most of the sweet-corn seed employed in the tests was grown on the experimental plots of the Wisconsin Agricultural Experiment Station. The field-corn variety, Golden Glow, was obtained from Dr. J. G. Dickson and N. P. Neal, of the Wisconsin station, the Leaming variety of field corn was grown by Dr. J. R. Holbert at Bloomington, Ill.; the unnamed variety of dent corn was purchased from a local seed firm.

The amount of diseased and surface-contaminated kernels in a sample of seed was determined by the poured-plate method. Most

of the inoculations were made by means of needle punctures at the base of the plant.

Isolations of the pathogene from the host tissue were made in the usual manner. The medium commonly employed for the isolation of the organism from diseased host tissue, when it was believed to be present in abundance and relatively free from contaminants, was nutrient dextrose agar. For making isolations from badly contaminated diseased material, a selective medium, described later in the paper, was employed. The identity of the organisms secured was determined both by cultural and pathogenicity tests.

Material for histological studies was collected from plants grown in the greenhouse and field and inoculated by the method already mentioned. Since it was found through isolation studies described later that not all of the kernels harvested from diseased corn plants harbored the bacteria within the kernel tissue, a method was employed by which the presence of the bacteria within the tissue of the kernel was established before the material was fixed. This method was as follows: The kernels which were supposed to be diseased were surface-sterilized and placed in sterile Petri dishes, one kernel in each dish. By means of a scalpel a bit of tissue from the tip of the kernel was cut off. The remaining portion of the kernel was removed to another container, labeled, and saved. The excised portion was then soaked in water in the dish and later crushed. Dilution plates were poured in the usual way. Only kernels from which the bacteria were recovered in this manner were used; the others were discarded. This method was based on the fact, established by Smith (*15*) and later confirmed in these studies, that the bacteria in the tissue of a diseased kernel are at the base of the kernel. Most of the kernels used for histological studies were in the milk stage, since it was found that such kernels were much easier to section.

The fixative most commonly employed for all diseased material was formal acetic alcohol. It destroyed the chondriosomes present in the plant tissue and obviated any possible confusion of these bodies with the bacteria. The fixed material was dehydrated in alcohol, treated with chloroform, and infiltrated and embedded in paraffin in the usual manner. Sections were made 10 to 30 microns thick. Flemming's triple stain was ordinarily employed.

SYMPTOMS OF THE DISEASE

Some symptoms not hitherto described, or only partly described, were observed in plants inoculated by means of needle punctures and grown in the greenhouse and in the field.

The appearance of water-soaked and discolored stripes along the veins of leaves of naturally infected or artificially inoculated plants has been noted by other investigators (*2*, *5a*, *7*, *9*, *15*) (fig. 1, *A*). The manner of their formation and development, however, as well as the microscopic symptoms of the affected tissue seem to deserve greater attention than they have yet received.

The development of these stripes a few days after puncture inoculations at the base of young leaves was found to be as follows: The first visible symptom, which usually appeared about 3 days after inoculation, was the yellowing of the vein and a slight discoloration of the parenchymatous tissue along the affected vein for some distance

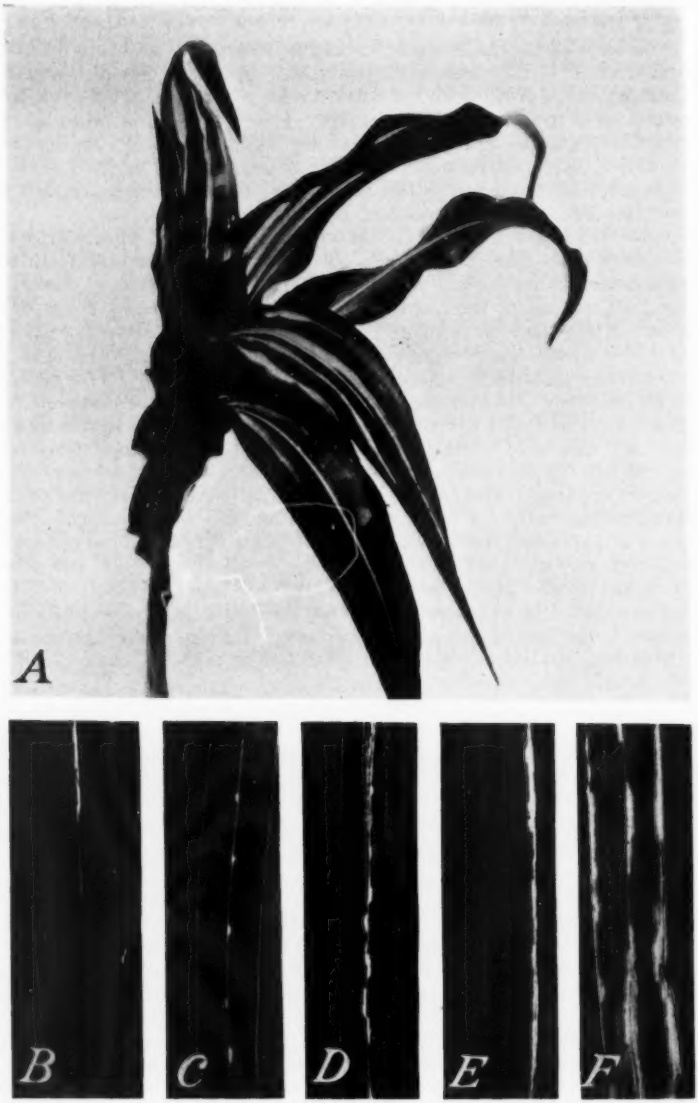


FIGURE 1.—Leaf symptoms of Stewart's wilt disease of corn: *A*, A diseased field-corn plant artificially inoculated in the stem with Stewart's wilt bacteria, showing several necrotic stripes along the veins; *B* to *F*, five stages in the development of these stripes; *B*, discolored veins which appear 2 or 3 days after inoculation; *C* and *D*, water-soaked, semicircular or irregularly shaped areas on either side of an affected vein; *E*, a stripe along an affected vein formed by the coalescence of the individual water-soaked areas; *F*, several water-soaked stripes which may unite to form a wider stripe upon the blade.

above and below the needle prick (fig. 1, *B*). This was usually followed a day or two later by the appearance here and there on either side of the affected vein of small, water-soaked, semicircular or irregularly shaped areas which appeared to have their centers at the vein (fig. 1, *C* and *D*). Subsequent histological studies showed that these water-soaked areas were near the points at which the bacteria escaped from the vessels into the parenchymatous tissue. Several of these areas coalesced shortly after they appeared and formed an irregular water-soaked stripe along the vein (fig. 1, *E*). Two or more stripes of the same blade in time might unite and produce a long discolored area along several of the veins until the whole blade became involved (fig. 1, *F*). The tissue of the semicircular water-soaked areas as well as that of the water-soaked stripes often lost its chlorophyll entirely, and it then appeared as a semitransparent membrane, which turned light brown with age. These areas were bordered by discolored tissue due to the partial disappearance of the chlorophyll, the discoloration being greatest near the border of the water-soaked region and gradually diminishing away from it (fig. 2, *E* and *F*). The discolored tissue immediately surrounding the water-soaked areas at first appeared slightly lighter than the normal green tissue. Later it turned dull gray, yellow, and light brown in the order named. The discoloration of the diseased tissue did not seem to advance in a regular line, as small dull-gray areas appeared intermingled with light-green or dark-green areas, giving the diseased tissue a mosaic appearance.

These stripes were not always continuous along an affected vein. Occasionally they were interrupted or gradually thinned out and appeared again farther along the same vein. The vascular tissue connecting the two water-soaked areas might or might not be discolored (fig. 2, *A*).

The same characteristic stripes appeared when the plants were inoculated at the root, stem, or midrib, but when the inoculation was made on the roots or stem the symptoms appeared after a relatively longer time. When the inoculation was made at the base of the midrib of an unfolding blade no symptoms were visible in the midrib, but from the points at which the numerous colorless vascular bundles traversing the midrib begin to separate from it and spread into the green tissue of the leaf, the characteristic water-soaked stripes appeared along the veins and gradually extended toward the tip of the leaf. In such cases the leaf appeared to wilt from the tip downward (fig. 2, *B* to *D*). Similar cases were observed when inoculations were made through the stem of the plant and the affected bundles extended for some distance through the midrib of the leaves.

The leaf symptoms seemed to vary with the host plant. On the leaves of the dark-green varieties of field corn the stripes were usually wider and more pronounced than on those of the lighter green varieties of sweet corn (fig. 2, *E*). Correspondingly, the leaves of the dark-green commercial variety of dent corn turned light brown and died sooner than those of Golden Bantam when both were grown and inoculated at the same time and under similar conditions.

Since the stripes appeared after wound-inoculation of young plants, they aided considerably in the early location of diseased plants, both in the greenhouse and in the field. When mature plants were inoculated these leaf symptoms were not observed. Similar leaf symptoms were produced when young corn plants were inoculated in the

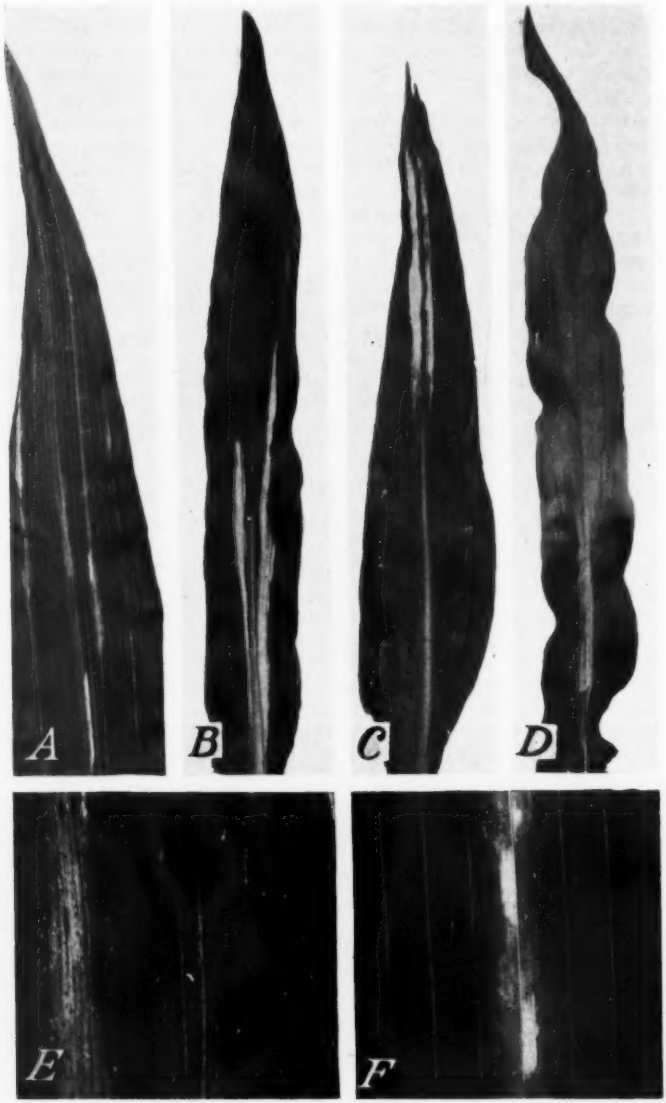


FIGURE 2.—Leaf symptoms of Stewart's wilt disease of corn: *A*, Stripes on a blade of Golden Bantam sweet corn in their various stages of formation. Leaf stripes are often discontinuous, as shown in this specimen. *B* and *C*, Leaves showing the characteristic stripes along veins which have separated from the midrib. The plants from which these leaves were taken were inoculated in the stalk. *D*, Same as in *B* and *C* but of a later stage, showing dried portion of the leaf extending from the tip downward. *E*, A wide, well-pronounced stripe on the blade of a dark-green variety of field corn. The numerous small dark spots on the stripe represent water-soaked areas in the parenchymatous tissue. ×3. *F*, Water-soaked areas adjoining an affected vein of a blade of a sweet-corn plant. Discolored tissue surrounds the water-soaked areas. ×4.

same manner with other bacterial cultures which differed in certain cultural and physiological characters (*3*, *5*) from the standard culture of *Phytomonas stewarti* used throughout this work.

Bacterial exudate was often observed upon the water-soaked or necrotic stripes of young seedling leaves. Most commonly it appeared in the form of small bright-yellow drops. In a few instances cream-colored drops of bacterial exudate were observed apparently exuding from the interior of the kernels. These kernels were found on anomalous tassels, part of which had assumed the form of pistillate spikes which produced fruit. As is usual in such cases, the kernels were not covered by husks, and there was no danger of their having been contaminated by the bacterial exudate commonly found on the inside of husks. After the exudate had been removed a slit in the kernel tissue, just beneath the bacterial drop, was visible.

Badly diseased cobs failed to develop normal kernels. Occasionally one side of a cob was found to be more seriously affected than the other. In such cases the kernels on one side of the cob developed to normal size while those on the other side were small, shriveled, or did not develop at all.

LIFE HISTORY OF THE CAUSAL ORGANISM IN RELATION TO PATHOGENESIS

The life history of *Phytomonas stewarti* in relation to pathogenesis was given especial attention. As stated above, four different phases in the life history of the organisms were considered—entrance, location, exit, and transmission.

ENTRANCE OF THE ORGANISM INTO THE HOST TISSUE

Several possible ways by which the bacteria might enter the host plant were studied. These were entrance (1) through the broken pericarp at the time of germination, (2) through unwounded roots, (3) through punctured roots, (4) through bruised and wounded roots from infested soil, (5) through wounds made by insects feeding upon the roots from infested soil, and (6) through stomata and water pores of young corn plants.

The ruptures which are made in the pericarp by the emerging primary root at the time of germination were tested as infection courts. Three tests were conducted, 2 in the laboratory and 1 in the greenhouse. In 1 laboratory test about 50 healthy kernels were surface-sterilized and placed upon the surface of hardened nutrient agar in Petri dishes, the embryo side up, 1 kernel to a dish. After the kernels had imbibed water and swelled overnight, and before the coleorhiza had emerged through the pericarp, a small drop of bacterial suspension was applied to the tip of each kernel. When the coleorhiza emerged through the pericarp, and the primary root tip appeared through the broken root sheath, there was already bacterial growth on the agar around and in contact with the basal edge of the kernel. As the primary root continued to grow and form branch roots and root hairs, the bacterial growth increased, finally completely surrounding all the roots and root hairs and partly enveloping the remnants of the kernel. The plumules appeared uncontaminated by the bacteria. Two weeks after germination no symptoms of disease appeared on the leaves of any of the seedlings. Subsequent histologic examinations of fixed specimens revealed masses

of bacteria between the pericarp and the tissue of the broken coleorhiza, between the coleorhiza and the epidermis of the primary root, and in the ruptured cells of the root sheath, but no bacteria inside the tissue of the young plant. The roots of some of the seedlings were then punctured with a needle. Four days later the characteristic water-soaked stripes appeared in the leaves of the punctured plants. In another trial, similar to the one just described, 42 healthy kernels were soaked in a bacterial suspension overnight and then placed on the surface of nutrient agar slants in large test tubes, where they grew in the absence of other organisms for 3 weeks. Although the bacteria grew profusely on the agar medium, none of the plants became infected. Subsequent isolations from the leaf tissue of each plant did not yield any bacteria. Similar greenhouse trials having the same object produced similar results.

Entrance of the pathogene into wound-free roots has not been found to occur under the conditions employed. To determine whether the bacteria gain entrance to the host plant through wounded roots and other injured underground parts of very young corn seedlings, two sets of experiments were conducted in the greenhouse. Uninfected seed was germinated under conditions which excluded any contact between the seedlings and the wilt bacteria. Four days after germination the seedlings were divided into two groups and given special treatment before they were planted in autoclaved soil. This treatment was as follows: Each seedling of the first group was painted by means of a camel's-hair brush at the primary root, at the broken coleorhiza, and at the lower part of the kernel, including the scutellum, with a suspension of the wilt organism. Some of these seedlings were punctured at the primary root by means of an inoculating needle and others were pricked at the scutellum by passing the needle through the region of the cotyledonary plate; the rest received no treatment. The seedlings of the second group were treated in a similar manner, except that sterile water instead of bacterial suspension was used. After these treatments all the seedlings were planted in holes dug for them in the bed. A week after planting, the characteristic water-soaked stripes appeared on the leaves of some of the seedlings that had been painted with bacterial suspension and punctured with a needle. Seedlings that had not been so treated showed no symptoms of the disease. The water-soaked streaks of the affected plants appeared to progress upward from the injury along the veins of the leaves.

Several experiments were conducted to determine whether infection could take place through wounds on the roots of plants growing in infested soil. The plants for these experiments were grown in boxes so constructed that special treatments and observations on the roots could be made. The boxes were about 20 inches long, 9 inches wide, and 13 inches deep. Two sides were removable. Under each of these removable sides there was fitted a glass plate to permit observation of the roots. Eighteen of the boxes were filled with soil, steamed, and seeded with bacteria-free corn, about 12 kernels to the box. When the plants were 2 to 3 weeks old the boxes were divided into 4 series for different treatments, as follows: The soil of the first series, consisting of 6 boxes, was infested with a suspension of Stewart's wilt bacteria, and the roots of the plants were wounded by running a dull scalpel through the soil. The soil of the second series of five boxes

was also infested with the bacteria, but the roots were left undisturbed. In the third series, consisting of four boxes, the roots were wounded and the soil was watered, but no bacteria were introduced. In the last series, consisting of one box only, the roots were left undisturbed and the soil free from wilt bacteria. Seven to fourteen days after the treatment the characteristic water-soaked stripes appeared on the leaves of some of the plants. The final results were taken about a month after the treatment. In the first series some plants in all of the 6 boxes were diseased, the percentage of diseased plants per box ranging from 18 to 100. In the second series, all of the plants in 3 of the boxes remained healthy but in 2 boxes there were 1 or 2 diseased plants, owing probably to accidental wounding of the plant roots in handling the boxes. In the boxes of the third and fourth series, where wilt bacteria were not introduced, no diseased plants were observed. These experiments were repeated 3 times, more than 500 plants in all being used.

In another experiment, white grubs, the larval stage of *Phyllophaga* sp., were used as agents for the wounding of the roots in soil artificially infested with the wilt bacteria. These insects were selected for wounding the roots because they commonly feed on roots of corn plants. In carrying out these trials the same materials and methods were used as in the experiments just described, except that instead of wounding the roots with a scalpel the white grubs, collected from under the sod, were placed on the surface of the soil of several boxes. A summary of the results of repeated trials with these insects showed that 50 percent of the plants became infected.

It was thought that the bacteria might enter through stomata and water pores of young corn plants. Accordingly, about 100 healthy kernels were surface-sterilized and planted on a bench in the greenhouse in autoclaved, insect-free soil. After the plants had reached the second- and third-leaf stage 60 were inoculated by placing a drop of bacterial suspension on the tip of each leaf. Thirty of the remaining plants were treated in a similar manner except that instead of bacterial suspension, sterile water was used. Four other plants were inoculated by placing a drop of the bacterial suspension at the base of the plant and a needle puncture made through it. Another 4 plants were similarly treated, except that sterile water was used instead of the bacterial suspension. The last three treatments served as controls. The temperature of the house at the time of these treatments and for a few days following was approximately 70° F. The plants were kept under observation for more than a month but no symptoms of disease were noted except on plants inoculated with the bacterial suspension by means of needle punctures. Before the plants were discarded a test was made for the presence of the bacteria within the plant tissue by plating out leaf tissue from a few of the plants which had previously had drops of bacterial suspension at their leaf tips. No wilt bacteria were recovered. A similar experiment was performed later in the season, and approximately similar results were obtained. In this experiment about 100 plants were grown in pots with sterilized soil. The inoculation was made when the plants were in the second- and third-leaf stage by placing 1 cc of bacterial suspension in the funnel formed by the uncurling leaves. The plants were kept in the humid atmosphere of the greenhouse for 5 days at a temperature of about 70° F. They were then taken out of the greenhouse.

No symptoms had appeared on any of the plants by the end of the third week after inoculation. Final observations were made at the end of 6 weeks, and it was found that 1 plant had a water-soaked stripe typical of Stewart's wilt disease. Since the stripe arose from below the soil level and appeared long after the usual incubation period for this disease, it was thought probable that infection had taken place through the roots at a time considerably after the plants were inoculated.

LOCATION OF THE BACTERIA IN THE HOST TISSUE

The location of the pathogene in the plant tissue has been studied by Smith (*15*), as previously mentioned. Many of his findings were verified in the present work and in some instances extended.

In the leaves the bacteria were located in the vessels of the vascular bundles, in large cavities adjoining disrupted bacteria-filled vessels, in the intercellular spaces, and substomatal cavities of the parenchymatous tissue, and as exudate, upon the surface of the blade.

In the vessels the bacteria were found in great masses, in many cases completely filling these structures. Gum material was also observed in the vessels. Its origin and the manner of its formation require further study. Often when a piece of diseased tissue was placed near the edge of a water drop, the drop would quickly become clouded with the bacteria, which would continue for several minutes to flow out from the vessels into the water drop.

In the parenchymatous tissue of the leaf, bacterial pockets or cavities were found adjacent to the openings in the walls of the vessels. These cavities were very numerous and appeared along one side, or more often, along both sides of a bacteria-filled vessel. They were of various sizes, reaching at times a diameter of one half millimeter or more and were invariably filled with large gummy masses of bacteria. These cavities seemed to correspond to the water-soaked semicircular areas, already described as occurring on either side of a vein a few days after inoculation (fig. 1, *C* and *D*, and fig. 2, *E* and *F*). The parenchymatous cells near the openings at the vessel wall were apparently destroyed by the outflowing mass of bacteria. There were some ruptured parenchymatous cells visible all around the bacterial cavities which were also filled with bacteria. Adjacent to these ruptured cells were several rows of other parenchymatous cells which appeared plasmolized and collapsed. These areas of diseased tissue were found to correspond to the discolored areas of the leaves described on page 753. The middle lamellae of some of these cells seemed to have been dissolved by the bacteria, which were found between the cells and in the intercellular spaces of the tissue (pl. 1, *A*). The discoloration of the cells gradually diminished as the number of bacteria between the cells decreased. These areas apparently corresponded to the dull gray or lighter green portions of the characteristic stripes on the leaves described above.

The exact manner in which the bacteria make their way out of the vessels into the parenchymatous tissue has not been determined.

In many of the substomatal cavities of the slightly discolored tissue adjacent to the necrotic leaf areas, masses of bacteria were observed with the microscope. Bacteria were also found on the characteristically wrinkled epidermal cells above these cavities. These bacteria, however, were not present in sufficient quantity to be seen

as exudate on the surface of the leaf. The bacterial exudate was found to come not only from the substomatal cavities but also directly from large, bacteria-filled vessels from which the bacteria made their way out as yellow drops visible to the naked eye through longitudinal slits in the walls of the vessels and the overlying leaf tissue.

In the husks the location of the bacteria was similar to that in the leaves. In some cases small drops of gumlike deposits, which took the blue of Flemming's triple stain, were found between the cells intermingled with the bacteria and in the intercellular spaces (pl. 1, *B*). Whether these deposits were produced by the bacteria or represented host reaction to injury, as is the case with some other diseases, was not determined.

In the stem, shank, and cob the bacteria were found in the vessels, in the large air spaces of some of the bundles, in masses in the disrupted pith tissue, and as an exudate on the surface of the shank. Many of the vessels were filled with bacteria. By making openings in the walls of the vessels the bacteria find their way into the large air space which most of the centrally located bundles of the stem have (pl. 1, *C*), and also into the adjoining sclerenchymatous and parenchymatous cells, where they progress between the cells. In the air spaces of the bundles the bacteria were found in great masses, considerably enlarging these spaces apparently by destroying the cells of the surrounding tissue.

In the tassel the bacteria were located in the vascular system of the rachises, of the rachillas, and of the glumes, in the delicate spiral vessels of the filaments, and in the parenchymatous tissue of the glumes. The location of the bateria in the rachises and the rachillas is similar to that in the stem and cob. In the glumes the bacteria were found in practically the same position as in the husks. Here also the bacteria occupied the substomatal cavities and the intercellular spaces adjacent to bacterial cavities caused by openings in the walls of the vessels. In some filaments the spiral vessels were almost completely occupied by bacteria, but no openings in the walls of the vessels were observed. Although these vessels are separated from the pollen chambers by only a few rows of cells, in the specimens examined no bacteria were observed in the pollen chambers. However, bacteria are sometimes found mixed with pollen.

Bacterial exudate similar to that found on diseased young seedling leaves was occasionally observed on the glumes. It exuded from the stomata located both above and below the level of the anthers.

Bacteria were isolated from the glumes, the anthers, and the pollen of diseased plants. These isolations were made from each part at 4 different times from material obtained from at least 3 different plants by removing the various parts of staminate spikelets of diseased plants under aseptic conditions. The isolations from the pollen were made by removing an anther from an open flower by means of sterile forceps and shaking it over a sterile Petri dish, after which a nutrient medium was poured in. It was not clear whether the bacteria were located in the pollen chambers or on the surface of the anthers. It is possible that the pollen had become contaminated by coming in contact with bacterial exudate oozing from the stomata of the glumes.

In the kernel the bacteria were located in the vascular system and parenchymatous tissue of the chalazal region, between the innermost

row of cells of the chalazal region and the marginal layer of the endosperm, and in the endosperm. The bacteria were found in the bands of spiral vessels which form a prolongation of the vascular system of the cob and extend into the tissue of the chalazal region and the pericarp. Some of these vessels were plugged with bacteria. Sometimes openings in the walls of the vessels occurred, resulting in the formation of cavities of various sizes (pl. 2, *A*). Often these cavities attained relatively large dimensions and were surrounded by collapsed cells. Frequently remnants of broken vessels were found mixed with the mass of bacteria (pl. 2, *B*). The number of these cavities varied in different kernels. In some kernels as many as 18 were observed in one section. All of these cavities were found in the parenchymatous tissue of the chalazal region, a few rows of cells from the endosperm. None were seen in the adjacent spongy tissue. In a few instances some of the parenchymatous cells of the chalazal region were badly ruptured, masses of bacteria lying among the broken cells (pl. 3, *A* and *C*). Through these ruptures the bacteria apparently found their way between the tissue of the chalaza and the marginal layer of the endosperm, where they were observed in masses embedded in slime. The further advance of the bacteria was followed through the ruptured aleurone layer to the starchy endospermic tissue where the bacteria lay between the cells in masses of slime (pl. 3, *B*).

EXIT OF THE BACTERIA FROM THE HOST PLANT

The exit of the bacteria from the host plant was studied by macroscopic observation and by histological and cultural technic.

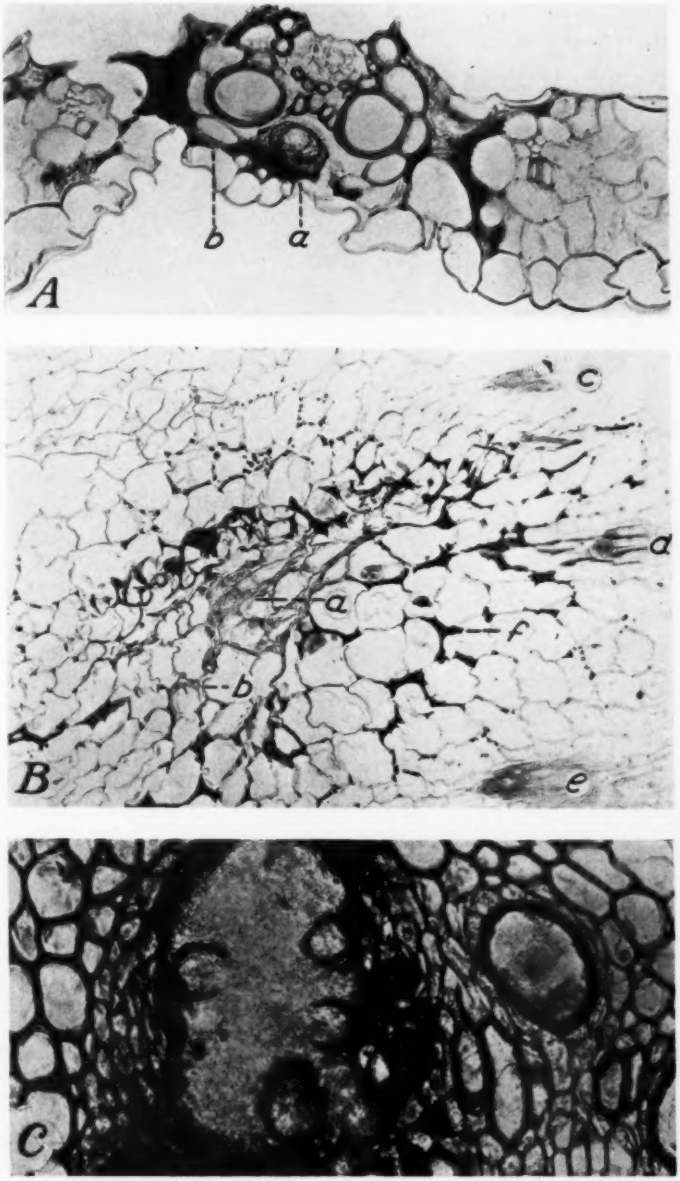
The exit of the bacteria from the host plant as exudate from diseased leaves, shanks, husks, glumes, and kernels has already been mentioned. This exudate is apparently washed from the diseased plant parts and carried with the rain water to other plant parts and also into the soil. The recovery of the bacteria from other plant parts, as well as from artificially infested soil and from dead plant material, was accomplished by employing a selective medium, described later in this paper.

The wilt organism was isolated from the water which accumulated between the blade and the ligule and at the base of young uncurling leaves of plants grown in the greenhouse and outdoors. Such isolations were made at two different times, three plants being used at a time. The aggregation of bacteria in these water drops was sometimes considerable, and was undoubtedly due to the washing down of bacterial exudate from diseased leaves by rolling dew drops and falling rain water. The role of this natural bacterial suspension in the initiation of the secondary cycles of the disease was not studied.

The bacteria were isolated without much difficulty from disintegrating plant parts taken from above and below the ground.

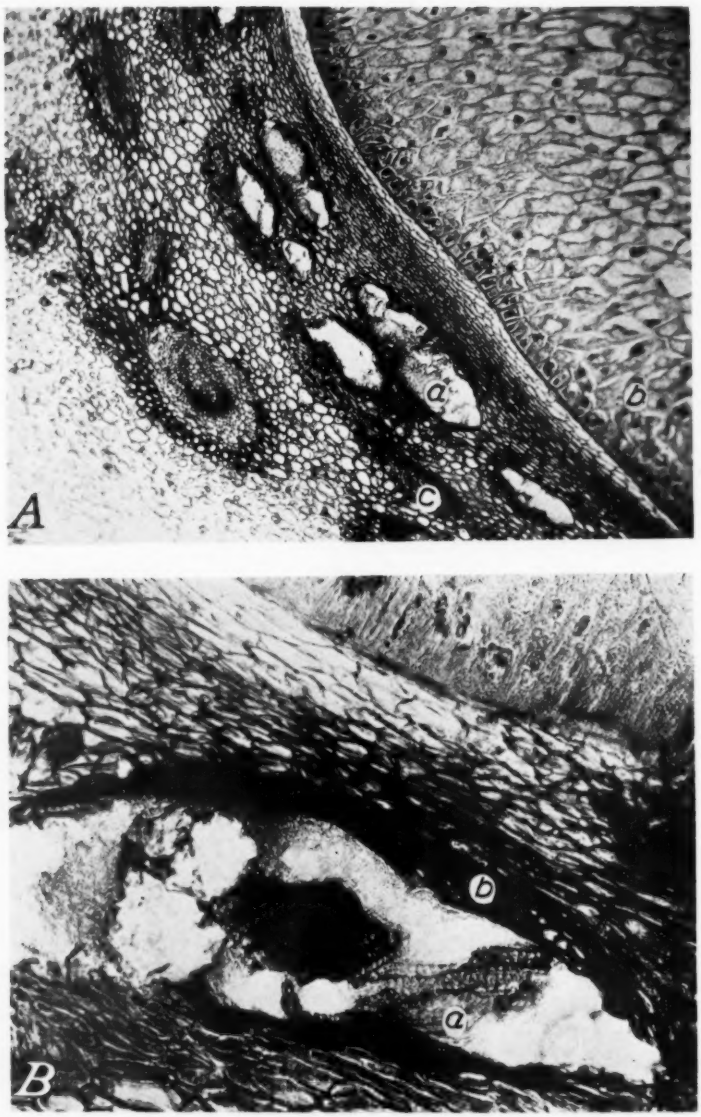
(1) From corn stubble of diseased plants which had overwintered in the field the bacteria were obtained in the following spring at planting time. The recovery of the bacteria from the stem tissues was made by crushing the tissues in a test tube of sterile water after which dilution plates were poured from the suspensions with the selective medium.

(2) From decaying roots and stems in 4 out of 6 cases the bacteria were recovered a few months after the crop had been harvested, by



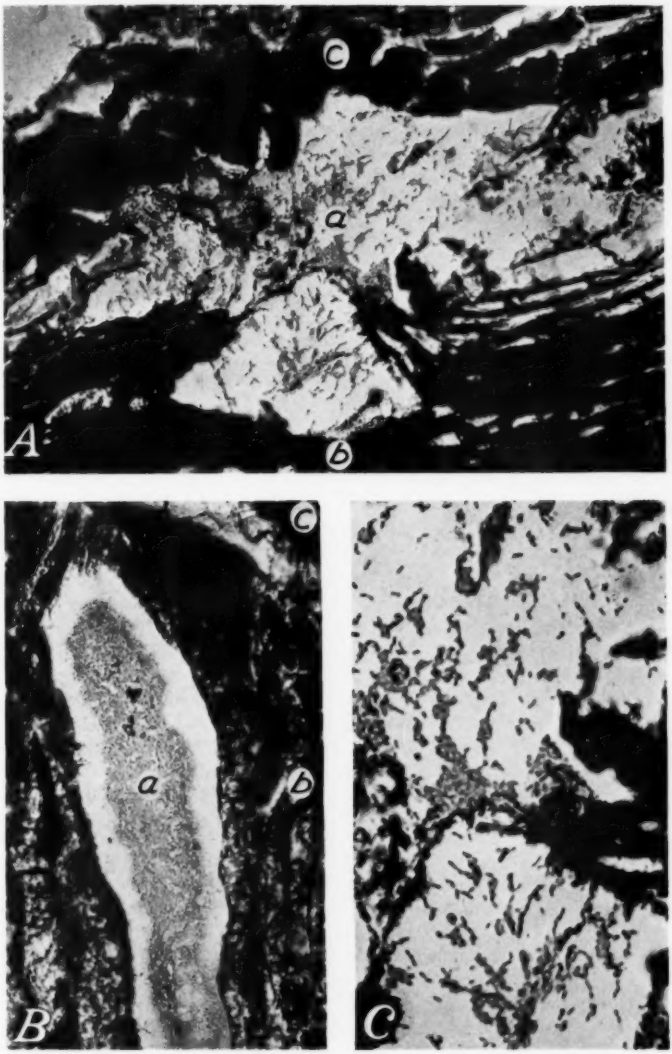
LOCATION OF STEWART'S WILT BACTERIA IN THE TISSUES OF CORN LEAF, HUSK, AND STEM.

A, Cross section through a water-soaked strip of a diseased leaf showing the general collapse of the tissues. The bacteria may be seen occupying one of the vessels as well as the intercellular spaces at *a* and *b*. × 340. *B*, Horizontal section through husk tissues beneath a drop of bacterial exudate. The bacteria may be seen in cavities in the parenchymatous tissue at *a* and between the cells at *b*. At the right of the illustration are three bascular bundles, *c*, *d*, and *e*, the middle one extending through the bacterial cavity. The wall of one of the vessels of this bundle had an opening near the cavity. Gumlike deposits may be seen in the intercellular spaces surrounding the bacterial cavity as at *f*. × 100. *C*, Cross section through the tissues of a diseased stem. The bacteria are shown in several vessels of a vascular bundle and in the cavity of the large air space of the bundle. × 310.



LOCATION OF STEWART'S WILT BACTERIA IN THE CORN KERNEL.

A, Bacterial cavities of various sizes, the largest shown at *a*, situated in the chalazal region of a kernel. The endosperm is shown to the right of these cavities, at *b*. Some of the vascular tissue of the kernel is shown at *c*, adjoining a bacterial cavity. × 80. *B*, Enlarged view of a bacterial cavity situated in the chalazal region of a corn kernel. Remnants of spiral vessels in the cavity are shown at *a*. The cells near the border line of the cavity at *b* appear to have collapsed. × 290.



LOCATION OF STEWART'S WILT BACTERIA IN THE CORN KERNEL.

A, Ruptured tissue in the chalazal region with bacteria in the cavity at *a*. The tissue of the chalaza appears at *b* and that of the endosperm at *c*. × 350. *B*, Bacteria, at *a*, embedded in slime between cells of the endosperm, which is shown at *b*. The tissue of the chalaza is shown at *c*. × 350. *C*, An enlarged portion from *B* showing individual bacterial cells together with remnants of ruptured host tissue. × 645.

using the selective medium. An attempt to isolate the bacteria from these decaying plant parts, by employing common laboratory media had previously been made without success.

(3) From disintegrating parts of six diseased kernels the bacteria were isolated a few days after the kernels had germinated in the soil. The survival of the bacteria in the kernel after germination was thought to be important in relation to the transmission of the disease through seed. In one of the laboratory experiments it was found that when diseased kernels were germinated on agar plates the bacteria made their exit through the ruptured tissue of the broken pericarp and completely surrounded the roots of the seedling, just as was the case with artificially contaminated seed, described above. Bacteria around the roots, if they do occur in the soil, might afford inoculum for the infection of the young plants after wounding. This phase of the problem requires further study.

(4) From artificially infested soil the bacteria were recovered several days after the infestation by employing the selective medium mentioned above.

TRANSMISSION OF THE DISEASE TO A NEW HOST

The transmission of the disease to a new host was tested in the following ways: (1) Through the soil, (2) through diseased seed, and (3) by transferring insects that had fed on diseased plants to healthy plants.

Experiments on soil transmission were not carried beyond the stage already described (pp. 756–757).

Experiments were conducted in the laboratory, greenhouse, and field to determine the role of infected seed in the transmission of the disease. In the laboratory 22 diseased kernels were germinated on nutrient agar plates. As expected, the bacteria from within the tissue found their way out and produced growth on the agar medium, completely surrounding the roots of the young seedlings. Two days after germination the seedlings were transplanted to two boxes filled with autoclaved soil, 11 plants being set in each box. The roots of the seedlings in one of the boxes were broken before planting; those of the remaining plants were left intact. Ten to 12 days after planting, 10 of the plants, the roots of which were broken, showed symptoms of disease, whereas only 2 of the plants in the other box became diseased. In these two cases it is possible that the roots of the plants were unintentionally broken at the time of transplanting. The number of plants used in this experiment was small because of a lack of infected seed.

The transmission of disease through infected seed was tested in the greenhouse by planting in autoclaved soil 100 kernels collected from diseased plants. Laboratory tests had shown that this particular sample of seed contained 9 percent of infected kernels. The plants were grown until they were 2 months old, but none showed any symptoms of disease. This experiment was carried out on a larger scale in the field by planting more than 500 kernels of seed collected from diseased plants of the previous year. This seed sample contained 4 percent of infected seed. The plants were examined from time to time until they reached maturity, but none was found to be diseased. The roots of a number of these plants were examined for root-chewing insects but none was found. These results are more or less in accord

with those obtained by Rand (*7*) in his greenhouse experiments with diseased seed. It appears very likely that wounding of the subterranean parts of the host plant at certain stages of its development by insects or other agencies may be an important factor in the transmission of the disease through seed. This phase of the problem requires further study.

Transmission of the disease by above-ground insects has been studied by Rand (*7*) and Rand and Cash (*9*). They reported that direct dissemination of the bacteria from leaves of diseased plants to leaves of healthy plants is accomplished through the agency of flea beetles and the 12-spotted cucumber beetle. Experiments on insect transmission were continued by the present writer, but in these experiments below-ground insects were employed.

During the summer of 1932 several thousand plants of several varieties of sweet corn obtained from various sources were grown at the Wisconsin Agricultural Experiment Station. Some of these plants showed the characteristic stripes of Stewart's wilt disease. Upon closer examination it was found that while the percentage of diseased plants was rather small (less than 1 percent), these plants adjoined each other, growing usually in groups of 2 or 3. Some of the diseased plants were removed, and it was found that on the roots or at the base of the stem of each plant from 1 to 3 insect larvae were feeding. Eleven of these were collected and later identified as the corn rootworm (*Diabrotica longicornis* (Say)). The stems of the diseased plants when split open showed the characteristic yellow bundles of Stewart's wilt disease. Subsequent isolations from the stems and leaves of these plants and from the larvae produced typical colonies of *Phytomonas stewarti*. The pathogenicity of these isolations was further established by inoculation tests. The role of these insects in the transmission of the disease was determined in the following manner. Each of the 11 bacteria-infested larvae was placed near the base of a young corn plant growing in the greenhouse. Care was taken not to disturb the roots of the plants. Each plant was then surrounded by a large metal cylinder which was thrust about 2 inches below the surface of the soil in order to confine the activities of the insect to one plant only. Six additional insects taken from apparently healthy plants were used in the same manner and served as controls. Bits of tissue of the healthy plants and washings from the control insects yielded no bacterial-wilt colonies on nutrient dextrose agar plates after the usual incubation period. The treated plants were examined daily for symptoms of disease. About 10 days after the treatment, 9 of the plants showed the characteristic leaf symptoms of bacterial wilt. The control plants showed no evidence of disease. Leaf tissue from 5 of the diseased plants and from 2 of the control plants was plated. Typical *Phytomonas stewarti* colonies were obtained in abundance from the tissue of the diseased plants but none from the control plants. During the attempt to recover the insects it was found that the insects on the 2 disease-free plants and 1 of the control insects had died. The rest of the larvae were alive and active, lodged at the base of each plant. The same experiment was repeated four times with the same lot of insects but on a new lot of healthy plants, some of which were grown in the special boxes mentioned previously. The results of these experiments were similar to those of the first. The lateness of the season prevented the use of a larger number of larvae for another series of trials.

DEVELOPMENT OF A SELECTIVE MEDIUM FOR THE DIRECT ISOLATION OF THE ORGANISM FROM THE SOIL

Attempts to isolate the bacteria directly from infested soil, or from badly contaminated plant parts, by employing common laboratory media, such as nutrient dextrose agar, potato agar, and yeast-infusion agar produced no positive results. The rate of growth of Stewart's wilt organism on these media was found to be considerably slower than that of many common soil organisms. Consequently it was outgrown and inhibited. An attempt was made, therefore, to develop, through physiological studies, a laboratory medium that would be more favorable for the growth of the wilt organism than for that of other bacteria and fungi commonly found in the soil. Accordingly, further studies were made in the growth requirements of the wilt organism. Since the object of these studies was to develop a selective medium, some of the tests by necessity were of a gross character, and were considered as preliminary to a more detailed physiological study of the bacteria to be made later. The studies were conducted along three different lines: (1) The influence of the hydrogen-ion concentration and the oxidation-reduction character of the medium upon the growth of the bacteria, (2) the nutrient requirements of the bacteria, and (3) the effect of the various inhibiting agents on the growth of the bacteria.

Certain bacteria and fungi commonly found in soil were run through some of the tests in parallel with the Stewart's wilt organism for the purpose of comparison. These organisms were *Phytomonas rhizogenes* Riker et al., *Bacillus subtilis* (Ehrenberg) Cohn, *Diplodia zeae* (Schw.) Lev., and an unidentified species of soil *Fusarium*.

HYDROGEN-ION CONCENTRATION AND OXIDATION-REDUCTION CHARACTER OF THE MEDIUM

Growth occurred in media having a wide range of hydrogen-ion concentration. A rough determination of the effect of hydrogen-ion concentration upon the growth of the bacteria was made in the usual manner by varying the reaction of nutrient broth containing 10 g of dextrose.

Abundant growth of the bacteria was observed from pH 6.0 to pH 8.0. On the acid side growth decreased at pH 5.5 but was still marked at pH 4.5. On the alkaline side growth decreased at pH 8.5. These determinations were repeated by using nutrient dextrose agar slants, and similar results were obtained.

Preliminary tests showed that highly oxidized media were favorable for the growth of the bacteria. The influence of the oxidation-reduction character of the medium was studied by employing a method described by Allyn and Baldwin (*1*). This method consisted in oxidizing and reducing certain restricted areas in Petri plate cultures by placing in them blocks of plain agar, adjusted to the proper pH, in which either oxidizing or reducing compounds had been incorporated. The influence of these blocks on the growth of the bacteria was indicated by the marked contrasts in the number of colonies appearing on the treated and untreated portions of the agar plate cultures.

Two media, A and B, were employed. Medium A had the following composition: Glycerol, 30 cc; sodium asparaginate, 10 g; sodium sulphate, 2.5 g; sodium chloride, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g;

and water to make a liter. The medium was adjusted to pH 7.0. Medium B was like medium A except that glycerol was omitted.

Three oxidizing compounds were used in the blocks in various concentrations. These were potassium permanganate, 0.1 percent; ferric ammonium citrate, 1 percent; and hydrogen peroxide, 0.001 and 0.002 percent. The reducing materials used were cysteine, 0.2 percent; and ferrous ammonium sulphate, 0.1 and 0.2 percent.

In these tests there was a marked increase in the number of colonies above and in the vicinity of blocks that contained oxidizing compounds and a diminution in the number of colonies above and around blocks that contained reducing compounds. The greatest stimulation of growth appeared above and in the vicinity of blocks that contained 1 percent of ferric ammonium citrate. The control blocks, containing plain agar only, influenced the growth of the bacteria unfavorably. Ferric ammonium citrate in the concentration employed appeared promising.

NUTRIENT REQUIREMENTS OF THE BACTERIA

The studies on the nutrient requirements of the bacteria included tests of various substances (1) as sources of carbon, and (2) as sources of nitrogen.

Sources of Carbon

A number of compounds were tested as sources of carbon. These included glucose, galactose, sucrose, maltose, lactose, starch, pectin, glycerol, and mannitol. The availability of these compounds for growth of the bacteria was tested by adding a definite quantity of each to a basic medium, which in this case was the sodium asparaginate mineral salts medium (B) already described. All of the compounds except glycerol were added to the amount of 1 percent; glycerol was added to the amount of 3 percent. After sterilization, each medium was adjusted to pH 7.0 and tubed under aseptic conditions. Two or three agar slants of each medium were then seeded with a 48-hour-old culture of the wilt bacteria grown on nutrient dextrose agar slants. For checks the last-named medium was used, as it was found to be very favorable for growth of the bacteria. The cultures were examined at the end of 2-day and 7-day periods. The results showed that most of the compounds used, including mannitol and glycerol, supported growth of the bacteria, which approximated in amount that on the nutrient dextrose agar slants. The relative availability of glycerol and mannitol to the bacteria was further tested by the poured-plate method. One cubic centimeter of a standardized bacterial suspension was placed in each of a number of Petri dishes and the medium poured in the plates. A subsequent count of the bacterial colonies showed the glycerol medium to be considerably more favorable for growth of the bacteria than the mannitol medium. Employing the same technic but using soil solutions instead of suspensions of the wilt organism, the two media were tested in relation to soil bacteria and fungi. The results showed that fewer of the soil micro-organisms grew on the glycerol medium than on the mannitol medium. Consequently glycerol was later selected as a source of carbon in the selective medium.

Sources of Nitrogen

The following salts were tested as sources of nitrogen: potassium nitrite, potassium nitrate, ammonium sulphate, sodium asparaginate, asparagine, and sodium taurocholate. Yeast infusion was also tried as a source of nitrogen. The basic medium in which these substances were incorporated had the following composition: Glycerol, 30 cc; sodium sulphate, 2.5 g; sodium chloride, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g; and distilled water sufficient to make a liter. The amount of each nitrogenous material added to the basic medium, with the exception of sodium taurocholate and yeast infusion, was 10 g to the liter. Three grams of the sodium taurocholate was added; yeast infusion was added in the proportion of 100 cc of a 10-percent infusion to 900 cc of the medium. A portion of the basic medium, without a nitrogenous compound, served as a check. The rest of the procedure in these trials was the same as that employed in the carbohydrate tests. The results showed that potassium nitrite did not favor the growth of the bacteria, potassium nitrate and sodium taurocholate produced a slight growth, and sodium asparaginate, asparagine, and yeast infusion produced good growth. The best growth was secured from ammonium sulphate. The other four organisms also grew on the ammonium sulphate medium but none grew so well as the wilt bacteria.

Influence of Various Inhibiting Agents on the Growth of the Bacteria

Several inhibiting agents were tested to determine what influence they might have on the growth of the bacteria. These agents were sodium taurocholate, sodium chloride, and various dyes. The first two compounds were tried singly or in combination in various concentrations in a medium having the following composition: Glycerol, 30 cc; ferric ammonium citrate, 10 g; sodium sulphate, 2.5 g; potassium phosphate (dibasic), 2.5 g; sodium chloride, 2.5 g; calcium chloride, 0.1 g; magnesium sulphate, 0.1 g; agar, 17 g; and water to make a liter. The dyes were tried in various concentrations in nutrient dextrose agar. The various materials were added in the desired concentrations in test tubes with a measured amount of melted agar medium, under aseptic conditions. Agar slants of the medium served as checks.

Sodium taurocholate did not inhibit the growth of the wilt bacteria when added to the medium to the extent of 0.3 percent. In fact, its presence in the medium stimulated the growth of the wilt bacteria, while it completely inhibited that of *Bacillus subtilis*. *Phytomonas rhizogenes* and the two fungi grew well on this medium but not so well as *P. stewarti*.

The effect of sodium chloride on the growth of the bacteria was tested. Smith (*15*) reported that the wilt organism grew promptly in +15 peptone bouillon containing 5 percent of this salt. In the present studies it was found that sodium chloride did not inhibit the growth of the wilt bacteria when added to the glycerol-ferric ammonium citrate-mineral salts-agar medium to the extent of 2.0 percent. On the other hand, the growth of *Phytomonas rhizogenes* and *Diplodia zeae* was completely inhibited when 1.5 percent of sodium chloride was added to this medium. The bacteria grew in higher

concentrations of sodium chloride when sodium taurocholate was present in the medium.

A number of dyes were found to have no inhibiting effect upon the growth of the wilt bacteria when employed in relatively low concentrations. The dyes tested were basic fucsin, crystal violet, malachite green, dahlia violet, methylene blue, and thionine. These were employed in concentrations of 1 to 10,000, 1 to 25,000, 1 to 50,000, and 1 to 100,000. Aqueous solutions of these dyes were made and 1 cc of each solution was added under aseptic conditions to 9 cc of nutrient dextrose agar adjusted to pH 7.0. Of the six dyes used, all but one—malachite green—permitted normal growth up to a concentration of 1 part of the dye to 10,000 parts of the medium. Malachite green inhibited the growth of the bacteria in a concentration of 1 to 100,000. The cultures were found to take up the colors of the dyes to a considerable degree. Because of this it was decided that no dye should be incorporated in the selective medium, for it was thought that the dye might obscure the characteristic yellow color of the wilt bacteria.

COMPOSITION OF THE SELECTIVE MEDIUM

The composition of the selective medium was determined on the basis of the results of the experiments just reported. The substances composing the medium were glycerol, 30 cc; ferric ammonium citrate, 10 g; sodium taurocholate, 3 g; sodium chloride, 15 g; sodium sulphate, 2.5 g; potassium phosphate (dibasic), 2.5 g; calcium chloride, 01. g; magnesium sulphate, 0.1 g; agar, 17 g; and distilled water to make a liter. The hydrogen-ion concentration of this medium was adjusted to pH 7.0.

The medium was prepared as follows: The agar was dissolved in the steamer in 400 cc of distilled water. The salts, with the exception of sodium taurocholate, were dissolved in a single container in 300 cc of water by gentle heating. Sodium taurocholate was dissolved in 200 cc of distilled water in the steamer. Following this, all the salt solutions, after the addition of the glycerol, were mixed with the melted agar and the liquid made up to a liter by the addition of distilled water. The medium was adjusted to pH 7.0 by the addition of approximately 17 cc of N/1 NaOH, tubed, and sterilized in the autoclave for 25 minutes at 15 pounds pressure.

EFFICIENCY OF THE SELECTIVE MEDIUM

The efficiency of the selective medium was determined by comparison with nutrient dextrose agar. Suspensions of wilt bacteria, soil, and a mixture of both were employed. The nutrient dextrose agar medium was selected as the standard of comparison because, of all the other common media tried, it was found to be most favorable for the growth of the wilt bacteria.

The relative efficiency of the two media was determined in the usual way by colony counts on comparable dilution plates. Colonies in all the plates appeared at the end of the third day after plating. The colonies on the nutrient agar plates were larger in diameter than those on the selective medium. However, the colonies on the selective medium were more raised. They were conspicuous owing to a more intense yellow color and a glistening appearance. The final counts of the plates were taken after an incubation period of 10 days,

by means of Frost's plate counter. The results showed that the number of colonies growing in the selective medium was almost twice as large as that of the colonies growing in the nutrient dextrose agar plates. In the case of the selective medium approximately one half of the colonies grew on the surface of the medium.

The influence of the selective medium on the soil micro-organisms that commonly appeared on the nutrient dextrose agar plates when attempts were made to isolate the wilt bacteria from the soil was tested in the following manner: Six soil suspensions of various concentrations were made. The heaviest of these suspensions was prepared by shaking thoroughly 50 g of rich greenhouse soil in a flask containing 500 cc of sterile water. The soil dilutions employed were 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, and 10^{-5}. One cubic centimeter of each suspension was placed in each of five Petri dishes by means of a pipette. The rest of the procedure was similar to that employed in the experiments described in the preceding paragraph. After an incubation period of 3 days at room temperature the plates of the first four suspensions containing nutrient agar were completely overgrown with bacteria and fungi; plates of the fifth suspension containing the same medium had an average of 200 colonies per plate. The plates of the suspensions in which the selective medium was employed had 2,000, 400, 40, 10, and 0 colonies, respectively. These results represent the average of three tests made at different times. From these results it appears that the selective medium is very unfavorable for the growth of a considerable number of soil bacteria and fungi.

When a certain number of the wilt bacteria were introduced in soil suspensions of various concentrations, the pathogene was recovered by means of the selective medium from some suspensions, but it was not recovered from the same suspensions when nutrient dextrose agar was used. The method of procedure in these tests was similar to that just described except that to each of the six soil suspensions an equal amount of a standardized suspension of Stewart's wilt bacteria was added. After an incubation period of 5 days about 5,000 colonies of the wilt bacteria appeared on the plates containing the selective medium of the third, fourth, fifth, and sixth suspension. The plates containing the nutrient agar, with the exception of these of the sixth suspension, were covered with various fungi and bacteria other than the Stewart's wilt pathogene.

The wilt bacteria were recovered from artificially infested soil a few days after their introduction, by using the selective medium. This was done in the following manner: Two boxes of soil were artifically infested with the wilt bacteria. One day after the treatment 50-g samples from each box were taken and suspended in 500 cc of sterile distilled water. The suspensions were shaken violently for 5 minutes. After the large soil particles had settled, part of each suspension was decanted and further diluted 100 times. Portions of 1 cc of each such suspension were placed in several Petri dishes and the melted medium poured in them. For checks, a similar set of plates was poured in which nutrient dextrose agar was used. The dishes were incubated at 28° C. and examined after 24 hours. The check plates were covered with bacterial and fungus growth, while the plates in which the selective medium was used were apparently free from any growth. On the fourth day after plating, numerous yellowish colonies

were observed on some of the selective-medium plates. It was later found that the colonies were mainly of three types, creamy white, yellow with orange-colored centers, and glistening buff colored, which in subsequent cultural and pathogenicity tests were found to be those of Stewart's wilt organism. The experiment was repeated by taking another sample of soil from each of the same boxes 5 days after its infestation. The bacteria were again recovered, although in smaller numbers, from the soil by using the selective medium. They did not appear on the plates in which nutrient dextrose agar was used.

Similar tests were made in which other bacterial wilt-producing cultures (*3*, *5*) were used. These bacteria showed no evidence of growth on the selective medium agar plates. Nor did they show growth in test tubes with liquid selective medium and Uschinsky's solution 3 weeks after they were transferred, although a typical culture of *Phytomonas stewarti* grew in these media profusely. It therefore appears that the selective medium cannot be used to advantage with all wilt-producing bacteria.

SUMMARY

Symptoms of Stewart's wilt disease of corn caused by *Phytomonas stewarti* (Smith) Com.S.A.B. are described for both sweet and field corn. The appearance of water-soaked stripes, which later turned yellow and light brown, along the veins after puncture inoculations of the roots, stems, and leaves of young plants was found to be characteristic of this disease. Bacterial exudate upon the surface of diseased leaves and glumes was observed.

The life history of the causal organism in relation to pathogenesis was given particular attention.

The bacteria were found to enter the host tissue through bruised and wounded roots in artificially infested soil, and through wounds made by white grubs, the larval stage of *Phyllophaga* sp., feeding upon the roots in infested soil. The bacteria were not found to enter through the broken pericarp at the time of germination nor through unwounded roots.

In the leaves the bacteria were found in the vessels of the vascular bundles, in large cavities adjoining disrupted bacterium-filled vessels, in the intercellular spaces and substomatal cavities of the parenchymatous tissue, and between cells in the place of the dissolved middle lamellae. The presence and the activities of the bacteria in these tissues caused discoloration, plasmolysis, and death.

In the husks the location of the bacteria was found to be similar to that in the leaves.

In the stem, shank, and cob the bacteria were found in the vessels, in the large air spaces of some of the bundles, in masses in the disrupted pith tissue, and as exudate upon the surface of the shank.

In the tassel the bacteria were located in the vascular system of the rachises, rachillas, and the glumes, in the delicate spiral vessels of the filaments, and in the parenchymatous tissue of the glumes. The bacteria were isolated from the anthers and pollen of diseased plants.

In the kernel the bacteria were located in the vascular system of the chalazal region, in cavities adjoining ruptured spiral vessels, between the outermost layer of the chalazal region and the aleurone layer, and between the cells of the endospermic tissue.

The wilt organism was isolated from exudate of diseased leaves, shanks, husks, glumes, and kernels, from the water which accumulated between the blade and the ligule, and at the base of young uncurling leaves. By employing a selective medium the bacteria were isolated from overwintered corn stubble, from decaying roots and stems, and from disintegrating parts of diseased kernels after the germination of the kernels in the soil.

The transmission of the bacteria to a new host through diseased seed took place when an avenue of entrance for the bacteria was provided by injuring the roots of the young seedlings. The corn rootworm, the larval stage of *Diabrotica longicornis* (Say), transmitted the disease from diseased to healthy plants.

A selective medium was developed with which the Stewart's wilt bacteria were recovered from badly contaminated plant parts and from artificially infested soil. This medium was developed in connection with studies on the physiology of the causal bacteria.

Other bacterial cultures, apparently different from the typical Stewart's wilt organism in some cultural and physiological characters, when inoculated into corn plants produced symptoms more or less resembling those of Stewart's wilt. The growth of certain of these cultures was not favored by the selective medium.

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INHERITANCE OF CHARACTERS IN RICE[1]

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INTRODUCTION

This paper deals with studies on the inheritance of the following characters in rice: Length of glumes, absence of ligules, brittleness, shattering, dwarfness, awns, color of awns, color of glumes, color of apiculi, and earliness.

LENGTH OF GLUMES

Most varieties of rice (*Oryza sativa* L.) have short (outer) glumes. The glumes usually vary in length from one fifth to one third of that of the lemma and palea. However, in some varieties the glumes are nearly as long, or quite as long, as the lemma and palea. These long-glumed varieties are reported to be resistant to lodging and shattering. They are not grown on a commercial scale in the United States.

Parnell et al. (*9, pt. 1*)[2] and Kato and Van der Stok, according to Ikeno (*4*), obtained in F_2 populations plants with short and long glumes in a ratio of 3:1. Chao (*2*), in a cross between a glutinous long-glumed variety and a common short-glumed variety, obtained in F_2 a ratio of 15 plants with short to 1 plant with long glumes. In this case short glumes were due to dominant duplicate genetic factors. Ramiah et al. (*10*), in a cross between short- and long-glumed varieties, obtained F_1 plants that had glumes intermediate in length between the parents. In the F_2 populations the parental and intermediate glume lengths were obtained. These investigators placed the F_2 plants with respect to glume length in three groups, namely, short, intermediate, and long, and by this arbitrary grouping they obtained approximately a 1:2:1 ratio. However, there was much variation in the length of the glumes of plants classified as intermediate. In this group the glumes varied in length from almost as short as the short-glumed parent to nearly as long as the long-glumed parent. This indicates that the plants classed as intermediate for glume length were not genetically similar and suggests that the difference between the short-glumed and the long-glumed varieties probably was due to multiple factors.

In 1926, Yosemite, a normally short-glumed variety, was crossed with "Nimai Kawa Mochi" and "Weitin," long-glumed varieties. The parents and F_1 plants were grown in 1927. The spikelets of F_1 plants from both crosses had normal, short glumes.

In the F_2 population of the cross Yosemite × Nimai Kawa Mochi 1,116 plants were grown to maturity. Of these, 831 plants had

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[2] Reference is made by number (italic) to Literature Cited, p. 782.

spikelets with short glumes and 285 plants had spikelets with long glumes. These numbers agree with a monohybrid 3:1 ratio, the deviation being only 6.00 ± 9.76 plants. Of the 1,047 Yosemite × Weitin F_2 plants 785 had short glumes and 262 had long glumes, the deviation from a 3:1 ratio being 0.25 ± 9.46 plants. In the total F_2 population of the two crosses there were 1,616 plants with short glumes and 547 with long glumes, the deviation from a monohybrid ratio being 6.25 ± 13.59 plants.

Fifteen random F_2 progenies from the cross Yosemite × Nimai Kawa Mochi and 16 progenies from the cross Yosemite × Weitin were grown in the F_3. Of the 15 progenies, 7 segregated for short and long glumes, 3 bred true for short glumes, and 5 bred true for long glumes. Of the 16 progenies, 7 segregated for short and long glumes, 4 bred true for short glumes, and 5 bred true for long glumes. The results for both groups are shown in table 1.

TABLE 1.—*Breeding behavior of F_2 progenies from the crosses Yosemite × Nimai Kawa Mochi and Yosemite × Weitin grown in F_3 at the Biggs Rice Field Station, Biggs, Calif., 1929*

Cross	Number of progenies	Number of plants with glumes—		Deviation from 3:1 ratio and probable error
		Short	Long	
Yosemite × Nimai Kawa Mochi	3	605		
	7	904	297	3.25±10.10
	5		837	
Yosemite × Weitin	4	704		
	7	964	346	18.50±10.54
	5		765	
Total for 14 segregating progenies		1,868	643	15.25±14.63

In the seven segregating progenies of each cross producing plants with short and long glumes the deviations from a 3:1 ratio were less than twice the probable error in all progenies, except for one in each cross. The results show that short glumes are dominant to long glumes and that the long-glumed and short-glumed varieties used in this study differ by a single genetic factor for this character.

ABSENCE OF LIGULES

In commercial rice varieties the ligules at the juncture of the leaf blade and sheath are usually well developed. A liguleless variety, "Tsutsuito," the only one known to the writer, was obtained in Japan in 1925. Tsutsuito is late-maturing, liguleless, and awnless. It was crossed with "Kotake," also late-maturing, and partly awned.

The F_1 plants had normal ligules and were partly awned like the male parent Kotake. An F_2 population, consisting of 1,024 plants, was grown in 1927. Of this number 796 plants had normal ligules and 228 were liguleless, the deviation from a 3:1 ratio being 28.00 ± 9.35 plants.

Fifteen random F_2 progenies were grown in the F_3 in 1928, of which 6, consisting of 989 plants, bred true for normal ligules, 5 segregated

and gave 655 plants with ligules to 195 without ligules, and 4, consisting of 725 plants, bred true for the liguleless character.

The five progenies that segregated in F_3 produced 655 plants with and 195 plants without ligules, the deviation from a 3:1 ratio being 17.50 ± 8.51 plants. According to Ikeno (*4*), Kato found that normal ligules were dominant to the liguleless character in rice. He obtained in F_2 3 plants with normal ligules to 1 without ligules.

BRITTLENESS

The culms and leaves of commercial rice varieties vary in strength and toughness. Under normal conditions some varieties do not lodge, whereas others lodge badly. Between these two extremes there are various degrees of resistance to lodging. However, all the commercial varieties are normal in that the straw possesses a certain degree of toughness or tensile strength and is somewhat resistant to complete breaking by bending or twisting. In contrast to the normal toughness of the culms and leaves of the commercial varieties is the extreme brittleness of the culms, leaves, and other parts of "Kama Irazu," a variety obtained in Japan. This variety does not lodge, although the stalks bend over under the weight of heavy panicles. However, in both the green and the mature stages the leaves and culms are so brittle that they break off at the slightest pressure. The writer was informed that the name Kama Irazu means "no need of a sickle in harvesting." This variety was so named because a standing plant gathered into the hand snaps off when given a slight twist. The panicles also are brittle and the spikelets fall off easily. In threshing, the lemma and palea often are removed and the kernel is easily broken.

In the cross Kama Irazu × Colusa (C.I.[3] 1600, a normal variety) the F_1 plants had normal (not brittle) stalks, leaves, and panicles. In the second generation there were 479 plants with normal stalks, leaves, and panicles to 156 plants with these parts brittle. The deviation from a 3:1 ratio was 2.75 ± 7.36 plants. The segregation into normal and brittle plants was very distinct. The ordinary green rice leaf often can be folded up without breaking into separate parts, but the brittle leaf is easily snapped off when bent. Owing to this fact it was possible to classify the plants even before they headed.

Twenty-five random F_2 progenies were grown in the F_3. Six of these, consisting of 292 plants, bred true for normal plants; 14 segregated, producing 453 normal plants to 142 brittle ones; and 5, consisting of 223 plants, bred true for the brittle character. The segregation of each of the 14 progenies agreed well with a ratio of 3 normal plants to 1 brittle plant, the deviation for the 14 progenies being 6.75 ± 7.12 plants.

Analyses were made of the straw and grain of Kama Irazu and of Colusa (C.I. 1600).[4] The straw of Kama Irazu contained less ash, silica, and crude fiber than that of Colusa, but a higher percentage of lignin, nitrogen, and moisture. The chemical data do not show the cause of brittleness in Kama Irazu, but this character may be due to cell structure or arrangement.

[3] C.I. denotes accession number of the Division of Cereal Crops and Diseases.

[4] These analyses were made by Dr. Jehiel Davidson, of the Bureau of Chemistry and Soils, U.S. Department of Agriculture.

SHATTERING

Varieties of rice differ greatly with respect to the tightness with which the grain is held on the panicle before and after maturity. In certain varieties the grain is held so tight that threshing is difficult. In others the grain shatters very easily, even when carefully handled. Between these two extremes there probably are varieties representing all degrees of tightness.

In the United States commercially grown rice varieties are more or less resistant to shattering but do not hold the grain so firmly that they cannot be satisfactorily threshed with modern machinery. Varieties that shatter too easily are not suited for harvesting with grain binders. In countries where rice is harvested by hand and threshed by treading and flailing, varieties that shatter rather easily are grown in preference to those that shatter less readily.

Takenouchi (*12*) reports that at the juncture of the rice grains and their supporting stalks there is a special tissue consisting of from 1 to 3 layers of lignified thin-walled cells. In rices that shatter easily these cells develop and dry up early, whereas in rices that shatter less easily they do not dry up so early.

According to Matsuura (*6*), Kato crossed a nonshattering rice with one that shattered easily. In the F_1 the plants were nonshattering, and in the F_2 the ratio of nonshattering plants to those that shattered easily was 3:1.

The Caloro and Colusa varieties, which are extensively grown in California, do not shatter easily if properly handled. These were crossed with Chinese varieties that shattered easily. The crosses were C.I. 7075×Colusa, C.I. 7078×Caloro, and C.I. 7389×Caloro. The F_1 plants of these crosses appeared to be intermediate. They did not shatter so readily as did the Chinese varieties, and yet they did not seem to hold the grain quite so firmly as did Colusa and Caloro.

Owing to various degrees of sterility in the F_2 plants of these crosses and to the failure of many individual plants to mature grain, only part of the F_2 population was suitable for a study of the inheritance of shattering.

On the basis of the ease or difficulty of stripping the grain from the mature panicles of plants standing in the field, it was not hard to determine relative resistance to shattering. Only a few trials were necessary to reduce this determination to a fair degree of accuracy. The plants from which it was easy to remove the seed were classed as easily shattered, whereas those from which it was rather difficult to remove the seed were classed as nonshattering.

In the F_2 population from the cross C.I. 7075×Colusa there were 29 nonshattering plants to 11 that shattered readily; from the cross C.I. 7078×Caloro there were 99 nonshattering plants and 46 that shattered readily; and from the cross C.I.7389×Caloro there were 249 nonshattering plants and 109 that shattered readily. In each cross these numbers agree reasonably well with a 3:1 ratio. The deviations from this ratio in the order given were 1.00 ± 1.85 plants, 9.75 ± 3.52 plants, and 19.5 ± 5.53 plants. The results indicate that in the varieties used in this study the nonshattering and shattering characters probably differ by a single genetic factor.

DWARFNESS

Dwarf varieties of rice often are grown at rice experiment stations. While they are of no economic importance, they are of interest to plant breeders. According to Ikeno (*4*), a dwarf rice plant was described by Iwasaki in 1828.

Parnell et al. (*9, pt. 2*) first reported on studies of the inheritance of dwarfness in rice. They found that dwarfness is a simple recessive to normal. The average height of the normal parent used in their study was 49.7 inches and that of the dwarf parent 27.9 inches. The average height of the normal segregates in F_2 was 50.5 inches and of the dwarf segregates 31.2 inches.

Sugimoto (*11*) states that dwarfness usually is a simple recessive to normal. He found a dwarf mutant in a true-breeding normal variety, Waseshinriki, which was a simple recessive to normal. Another dwarf appeared as a mutant in a true-breeding strain isolated from a hybrid. This dwarf was dominant to normal and in the F_2 segregation produced 3 dwarf plants to 1 normal.

Akemine (*1*) crossed the normal variety Akage with the dwarf varieties "Daikoku" and "Ebisu." In the F_2 segregation both crosses produced 5 normal plants to 1 dwarf. In a cross between the dwarf varieties Daikoku × Ebisu the F_1 was normal, and the segregation in F_2 gave 9 normal to 3 Daikoku to 3 Ebisu to 1 new very short dwarf type, Kodaikoku. Akemine assumed that the genetic constitution of normal was *AABB*, Ebisu *AAbb*, Daikoku *aaBB*, and Kodaikoku *aabb*.

Nagai (*8*) reported a dwarf mutant that was recessive to normal. This mutant showed a peculiar mode of inheritance in that in successive generations mutant individuals gradually increased in proportion to normals.

The writer obtained in Japan seed of a dwarf variety known as "Daikokune." This variety has short, rather wide, erect leaves, short culms, and a short, compact panicle that looks more like a spike of wheat than a panicle of rice. It is partly awned and the kernel is short and round and enclosed by a rather coarse hull. The cross Yosemite × Daikokune was made in 1927. Yosemite is an early-maturing, awnless variety of normal height.

The F_1 plants were intermediate in maturity, partly awned, normal in appearance, and somewhat taller than the normal parent. The average height of the normal parent was 38 inches, of the dwarf parent 14 inches, and of the F_1 plants 43 inches. The average length of 10 panicles of the normal variety was 4.7 inches, of the dwarf parent 2.3 inches, and of the F_1 plants 4.6 inches.

The F_2 population consisted of 677 plants that were grown to maturity, of which 547 were normal and 130 were dwarf. Segregation into the two groups was very distinct. The deviation from a 3:1 ratio was 39.25 ± 7.60 plants, or more than five times the probable error, whereas the deviation from a 13:3 ratio was only 3.0625 ± 6.85 plants. However, the segregation of random F_2 progenies grown in F_3 shows that the 3:1 ratio is correct.

In the F_2 population the average height of 100 normal segregates was 37.4 inches and the average height of 100 dwarf segregates was 16.9 inches. The average height of 42 plants of the normal variety was 32.6 inches and the average height of 42 dwarf plants was 13.9 inches. The normal and dwarf segregates in F_2 exceeded the normal and dwarf parent varieties in average height.

Of 25 random F_2 progenies grown in 1930, 7, consisting of 367 plants, bred true for normal stature, 15 segregated for normal and dwarf plants, and 3, consisting of 86 plants, bred true for dwarf plants. Each of the 15 segregating progenies produced normal and dwarf plants in numbers that agreed well with a 3:1 ratio. The total for the 15 progenies was 598 normal to 181 dwarf plants, the deviation from a 3:1 ratio being 13.75 ± 8.15 plants. In the varieties used in this study dwarf character was a simple recessive to normal.

AWNS

Rice varieties may be classed as fully awned, partly awned, and awnless. In fully awned varieties all spikelets are awned but the awns often vary in length. In partly awned varieties awned and awnless spikelets are present on the same panicle. In true awnless varieties the awns are absent and do not develop under any conditions. In some varieties the main panicle may be awnless, whereas some of the spikelets on branch culms and later tillers may be partly awned. The extent of development of the awns in rice is controlled by climatic conditions and soil fertility as well as by genetic factors. In some crosses it is difficult to separate the awn types because climatic conditions and soil fertility have such a marked influence upon their development.

Chao (*2*), Hoshino (*3*), Jones (*5*), Mendiola (*7*), Nagai (*8*), Yamaguchi (*13*), and Kato and Van der Stok, according to Matsuura (*6*), have reported that in F_1 awns are dominant or partially dominant to the awnless character in rice. A segregation in F_2 of 3 awned or partly awned plants to 1 awnless plant has been reported by Jones (*5*), Nagai (*8*), and Chao (*2*), and a ratio of 9 fully awned to 6 partly awned to 1 awnless plant by Jones (*5*). The awned group in the 15:1 ratio reported by Chao (*2*) were designated as fully awned, mostly awned, and rarely awned plants.

In the few crosses between fully awned and awnless varieties that have been studied by the writer, three groups of awned plants have been observed in the F_2 segregations. These are fully awned plants, partly awned plants with awns about the same length as in fully awned plants but present only on part of the spikelets on a panicle, and partly awned plants with short awns often confined to the spikelets near the tip of the panicles. The plants in this last group may be referred to as tip-awned. It appears that fully awned varieties often differ from awnless varieties by two independent genetic factors. If this is true, the fully awned group from a cross between a fully awned and an awnless variety should differ from the partly awned and tip-awned groups by a single genetic factor, and the partly awned group should differ from the tip-awned group by two genetic factors. Data in support of this view are reported herein.

AWNLESS × FULLY AWNED

In the cross Colusa (awnless) × Aikoku (fully awned) the F_1 plants were partly awned. In the F_2 segregation 607 plants were classed as fully awned, partly awned, and tip-awned to 71 awnless plants. The deviation from a 15 : 1 ratio in this case is 28.63 ± 4.25 plants. This deviation is very large.

Twenty-five random F_2 progenies were grown in F_3. Five progenies produced 225 fully, partly, and tip-awned plants to 13 awnless

plants. These numbers agree with those expected in a 15 : 1 ratio, the deviation being 1.88 ± 2.52 plants. Six progenies, consisting of 309 plants, produced fully awned, partly awned, and tip-awned plants in what appeared to be a ratio of 3 fully awned to 1 partly awned, and 3 partly awned plants to 1 tip-awned. However, the awn types in this cross could not be separated with certainty. Ten progenies produced no fully awned plants, but 347 partly awned and tip-awned plants to 120 awnless plants, deviating from a 3 : 1 ratio by 3.25 ± 6.31 plants. One progeny, consisting of 46 plants, appeared to be fully awned, and three progenies, consisting of 119 plants, appeared to produce only partly awned or tip-awned plants. The number of random F_2 progenies was too small to yield all the possible segregations and true-breeding groups that were expected. However, the results suggest that the fully awned variety Aikoku probably differs from the awnless variety Colusa by two main genetic factors for awns.

PARTLY AWNED × FULLY AWNED

In the cross Caloro (partly awned) × Butte (fully awned) the F_1 plants were fully awned. The segregation for awns was not studied in F_2, but a study was made of the segregation of 11 random F_2 progenies grown in F_3. Each of the 11 F_2 progenies and the total of the 11 progenies produced fully awned and partly awned plants in a ratio of 3 : 1. In the 11 F_2 progenies there were 393 fully awned to 99 partly awned plants, the deviation from a 3 : 1 ratio being 24.00 ± 6.48 plants. This deviation, which is more than three times the probable error, was probably due to errors in classifying the plants resulting from the natural variability of awn development. In the individual progenies there were 3 small plus deviations, 7 minus deviations, and 1 progeny with no deviation from the 3 : 1 ratio.

PARTLY AWNED × AWNLESS

Colusa, Chinese Originario, Maratelli, and Yosemite, awnless varieties, were used as female parents in crosses with Wataribune and Caloro, partly awned varieties. Yosemite, Chinese Originario, and Colusa were crossed with Wataribune, Yosemite, and Chinese Originario, and Maratelli was crossed with Caloro. In each of the six crosses the F_1 plants were partly awned.

TABLE 2.—*F_2 segregation of crosses between awnless and partly awned rice varieties grown at the Biggs Rice Field Station, Biggs, Calif., 1929*

Cross		Number of F_2 plants			Deviation from 3 : 1 ratio and probable error
Female, awnless	Male, partly awned	Partly awned	Awnless	Total	
Yosemite	Wataribune	450	199	649	30.75±7.44
Chinese Originario	do	421	121	542	14.50±6.80
Colusa	do.*	251	94	345	7.75±5.42
Chinese Originario	Caloro	473	161	634	2.50±7.35
Yosemite	do	423	178	601	27.75±7.16
Maratelli	do	484	184	668	17.00±7.55

* Grown in 1930.

The F_2 segregation of the six crosses is shown in table 2. In all crosses the number of partly awned and awnless plants agrees well with a 3 : 1 ratio, except for the crosses Yosemite × Wataribune and

Yosemite×Caloro. The deviations in these crosses are large and suggest the possibility of a modifying factor in the Yosemite variety.

Twenty-five random F_2 progenies from the crosses Yosemite×Wataribune, Chinese Originario×Wataribune, Colusa×Wataribune, and Chinese Originario×Caloro were grown in F_3. The breeding behavior of these progenies is shown in table 3.

TABLE 3.—*Breeding behavior in F_3 of F_2 progenies from crosses between awnless and partly awned rice varieties grown at the Biggs Rice Field Station, Biggs, Calif., 1930*

Cross		Number of F_2 progenies grown in F_3	Number of F_3 plants		Deviation from 3:1 ratio and probable error
Female, awnless	Male, partly awned		Partly awned	Awnless	
Yosemite	Wataribune	3	142		
		[a] 16	605	153	36.5±8.04
		4		197	
Chinese Originario	do	6	258		
		[b] 13	494	136	21.50±7.33
		4		170	
Colusa	do [b]	7	349		
		11	446	168	14.50±7.24
		[c] 7		360	
Chinese Originario	Caloro	11	550		
		[d] 11	429	121	16.50±6.85
		2		100	

[a] 2 F_2 progenies did not agree with 3:1 ratio in F_3 in these 2 crosses.
[b] Grown in 1931.
[c] 1 spikelet on each of 4 panicles appeared to have a tip awn.
[d] 1 F_2 progeny did not agree with 3:1 ratio in F_3.

In each cross three or more F_2 progenies bred true for partly awned plants and two or more bred true for awnless plants. Of the F_2 progenies that segregated in F_3 all produced partly awned and awnless plants in a ratio of 3:1, except 1 or 2 progenies in each cross. The failure of some segregating F_2 progenies to give the expected 3:1 ratio may have been due to errors in classification or possibly to natural crossing in F_1, as the F_1 plants were not bagged. The fact that nearly all the F_2 progenies gave the expected 3:1 ratio in F_3 indicates that the partly awned and awnless varieties used, with the possible exception of Yosemite, probably differ by a single genetic factor.

The data on the inheritance of awns in crosses between an awnless and a fully awned variety, a partly awned and a fully awned variety, and awnless and partly awned varieties indicate that the fully awned varieties differ from Colusa, awnless, by two independent genetic factors. Butte, fully awned, appears to differ from Caloro, partly awned, by a single genetic factor, and Caloro and Wataribune, partly awned, appear to differ from the awnless varieties by a single genetic factor.

COLOR OF AWNS, GLUMES, AND APICULI

AWNS

Aikoku has red awns, glumes, and apiculi, whereas Colusa is awnless and has green apiculi and glumes, which are straw-colored at maturity. In the cross Colusa×Aikoku the F_1 plants had red awns, glumes, and apiculi. In the F_2 population 454 plants had red awns and 153 plants had green awns. These numbers agree well with a 3:1 ratio, the deviation being only 1.25 ± 7.20 plants.

Of 25 random F_2 progenies grown in F_3, 4, consisting of 185 plants, bred true for red awns; 13 produced 392 plants with red awns to 126 plants with green awns; 3 progenies, consisting of 133 plants, bred true for green awns; and of 5 remaining F_2 progenies, 3, consisting of 141 plants, segregated for red-awned and awnless plants with red glumes and apiculi, and 2, consisting of 97 plants, segregated for green-awned and awnless plants with green glumes and apiculi. Each of the 13 F_2 progenies that segregated for red and green awns did so in a ratio of 3:1. In the 13 progenies as a group the deviation from a 3:1 ratio was 3.50 ± 6.65 plants, which is a satisfactory fit.

Caloro is partly awned and has green awns and glumes. In the cross Caloro × Aikoku the F_1 plants had red awns and glumes. In the F_2 population there were 402 plants with red awns to 261 plants with green awns. These numbers are in fair agreement with a 9:7 ratio. The deviation was 29.0625 ± 8.62 plants. Of the F_2 population, 663 plants were classed as awned and 15 as awnless. One random F_2 progeny, which was classed as awnless in F_2, was partly awned in F_3. Therefore, it is probable that other plants classed as awnless in F_2 carried factors for awns but that owing to environmental conditions no awns were visible.

Of the random F_2 progenies grown in F_3, 4, consisting of 202 plants, bred true for red awns; 5 produced 143 plants with red awns to 105 plants with green awns; 9 produced 269 plants with red awns to 90 plants with green awns; and 7, consisting of 301 plants, bred true for green awns. The deviation from a 9:7 ratio for 5 of the F_2 progenies was 3.50 ± 5.27 plants, which is a satisfactory fit. The deviation from a 3:1 ratio for 9 of the F_2 progenies was 0.25 ± 5.53 plants, or almost a perfect fit.

Butte is a fully awned variety with red awns. In the cross Caloro × Butte the F_1 plants had red awns. In the segregation in F_2, 271 plants had red awns to 251 plants with green awns. In this case the deviation from a 9:7 ratio is 22.625 ± 7.64 plants. Twenty random F_2 progenies were grown in F_3. Four, consisting of 198 plants, bred true for red awns; 4 produced 91 plants with red awns to 63 plants with green awns, or in a ratio of 9:7; and 12 progenies, consisting of 560 plants, bred true for green awns.

In the cross Colusa × Aikoku the difference in awn color apparently was due to a single genetic factor, whereas in the crosses Caloro × Aikoku and Caloro × Butte the difference appeared to be due to two complementary genetic factors. The results indicated that Colusa has one complementary genetic factor in common with Aikoku and Butte (*5*). However, Caloro appears to differ in awn color from Butte and Aikoku by two complementary genetic factors.

GLUMES AND APICULI

Aikoku has red glumes and Colusa has green glumes. In the cross Colusa × Aikoku the F_1 plants had red glumes. Segregation for color of glumes in F_2 gave 504 plants with red glumes to 174 plants with green glumes. The deviation from a 3:1 ratio is 4.50 ± 7.60 plants.

Of the 25 random F_2 progenies grown in F_3, 7, consisting of 326 plants, bred true for red glumes; 13 produced 460 plants with red to 163 plants with green glumes; and 5, consisting of 230 plants, bred true for green glumes. Each of the 13 F_2 progenies that segregated

for glume color produced plants with red and green glumes in a ratio of 3:1. The deviation from this ratio for the 13 F_3 progenies was 7.25 ± 7.29 plants.

In the cross Caloro × Aikoku, green by red glumes, the F_1 plants had red glumes. In F_2 the segregation for color of glumes gave 410 plants with red glumes to 268 with green glumes. The deviation in this cross from a 9:7 ratio was 28.63 ± 8.71 plants.

In the crosses Colusa × Aikoku and Caloro × Aikoku the color of the apiculi was always the same as that of the glumes and appeared to be controlled by the same genetic factor or factors.

The red glumes of Aikoku differ from the green glumes of Colusa by a single genetic factor and from the green glumes of Caloro by two dominant complementary genetic factors. The same is true of the color of the apiculi of these varieties. In all hybrids studied the color of the apiculi of the lemma and palea was the same as that of the awns. However, the writer knows of one variety with red apiculi, the awns of which are green at maturity.

COLOR OF LEMMA AND PALEA FURROWS

The cross Yosemite × Weitin, used in the study of glume length, also presented an opportunity to study lemma and palea furrow color. In Weitin the lemma and palea furrows are yellowish brown, and in Yosemite they are straw-colored at maturity. In F_1 plants of the cross Yosemite × Weitin the furrows were yellowish brown. Of 1,047 F_2 plants 834 had yellowish-brown and 213 had straw-colored lemma and palea furrows. These numbers agree well with a 13:3 ratio, with yellowish brown dominant to straw color, the deviation from the 13:3 ratio being 16.69 ± 8.52 plants.

Segregating F_3 progenies were not classified for color of the lemma and palea furrows. It was observed, however, that the 7 F_2 progenies segregating for short and long glumes also segregated for color of lemma and palea furrows. Three of the 4 F_3 progenies that bred true for short glumes had yellowish-brown lemma and palea furrows, and the other family segregated for furrow color. One of the 5 F_3 progenies that bred true for long glumes had yellowish-brown lemma and palea furrows, 1 progeny segregated for furrow color, and 3 progenies bred true for long glumes with straw-colored lemma and palea furrows.

The factors for yellowish-brown color of the lemma and palea furrows and the factor for short glumes appear to be inherited independently, as shown in table 4.

TABLE 4.—*Phenotypes and observed and calculated number of F_2 plants in each group from the cross Yosemite × Weitin, grown at the Biggs Rice Field Station, Biggs, Calif., 1928*

Phenotype [a]	Observed	Calculated	$\frac{(O-C)^2}{C}$
Short glumes, yellowish-brown lemma and palea furrows	637	638.04	0
Short glumes, straw-colored lemma and palea furrows	148	147.24	0
Long glumes, yellowish-brown lemma and palea furrows	197	212.68	1.16
Long glumes, straw-colored lemma and palea furrows	65	49.08	5.16
	1,047	1,047.04	

$P = 0.0989$ $\chi^2 = 6.32$.

[a] Expected ratio 39:9:13:3.

EARLINESS

In the cross Colusa (an early variety) × Wataribune (a late variety) the F_1 plants and Wataribune matured on October 4 and Colusa on September 19. In the F_2 population there appeared two quite distinct groups with respect to maturity, namely, early and late. On September 6 the early plants were fully headed and starting to mature whereas the late plants were just coming into full heading. On this date the F_2 plants were classified as early and late on the basis of stage of development. There were 264 late to 81 early plants, the deviation from a 3:1 ratio being 5.25 ± 5.42 plants.

Twenty-five random F_2 progenies were grown in F_3. Six progenies, consisting of 232 plants, bred true for late maturity; 12 progenies that segregated for late and early plants produced 485 late to 186 early maturing plants; and 7 progenies, consisting of 341 plants, bred true for early maturity. The deviation from a 3:1 ratio for the 12 segregating progenies was 18.25 ± 7.57 plants.

Other studies and observations on the inheritance of earliness in rice have indicated that multiple factors often are involved. It is of interest, therefore, to note the simple segregation in this case. The factorial situation for this as well as for other characters in rice varies with the varieties used.

SUMMARY

The rice crosses used in these studies were grown for three generations, except those used in a study of shattering.

In the varieties studied short glumes were dominant to long glumes.

The characters normal ligule, normal culm strength, and nonshattering were found to be simple dominants to absence of ligules, brittleness of culm, and shattering of grain, respectively.

The dwarf variety studied was recessive to the normal.

In the crosses awnless × fully awned, partly awned × fully awned, and partly awned × awnless varieties, the data indicate that the fully awned varieties used differ from awnless varieties by two genetic factors, fully awned varieties differ from partly awned by a single genetic factor, and partly awned varieties differ from awnless varieties by a single dominant genetic factor.

In these studies red color in the awns, glumes, and apiculi was dominant to green (straw color). Ratios of 3:1 and 9:7 were obtained, which show that the color was due to a single genetic factor and to two complementary genetic factors.

In Weitin brownish-yellow lemma and palea furrows appeared to be inherited independently of long glumes. The brownish-yellow color of lemma and palea furrows was dominant to straw color and appeared to be due to two genetic factors resulting in a 13 : 3 ratio.

Plants from the cross Colusa × Wataribune were obtained in the ratio of 3 late to 1 early.

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IDENTITY AND HOST RELATIONS OF THE ELSINOE OF LIMA BEAN [1]

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INTRODUCTION

This paper presents the results of inoculation tests on legumes with the fungus causing scab of the lima bean (*Phaseolus lunatus macrocarpus* Benth.). This organism was tentatively identified [2] as *Elsinoe canavaliae* Rac., causing scab of *Canavalia* in the Orient. The plants inoculated were wild or naturalized lima bean, known in Cuba as "frijol caballero"; several common horticultural varieties of lima bean, including Fordhook Bush, Henderson Bush, Henderson Pole, and Challenger Pole; and some other leguminous plants, namely, *Calopogonium caeruleum* (Benth.) Hemsl., *Canavalia gladiata* (Jacq.) DC., *C. ensiformis* (L.) DC., *Dolichos lablab* L., *Phaseolus vulgaris* L., *Pisum sativum* L., and *Stizolobium deeringianum* Bort. The object of the investigations was to obtain additional data on the host range of the organism, which would be of value taxonomically and otherwise, and also to determine some of the environmental conditions necessary for infection of the lima bean.

MATERIALS AND METHODS

The inoculations were made at the Agricultural Experiment Station, Santiago de las Vegas, Cuba, from 1930 to 1932 by the senior writer. The plants of *Calopogonium caeruleum* and some of those of *Canavalia ensiformis* were inoculated on the station grounds where they were growing. The other inoculations were made in the plant-quarantine house. The sides of this house were protected with panels of fine copper-wire screening, which made it practically insect-proof but not fungus-proof. No infected lima-bean fields were located nearby, and no accidental infections took place on any of the plants under observation—either those inoculated or those used as controls. In fact, the conditions were such that there was no spread of the disease from the primary infections resulting from the artificial inoculations. There was no appreciable condensation of dew on the plants, and, moreover, they were largely protected from wind and rain—factors believed to greatly favor the dissemination of the fungus in the field.

The legumes inoculated were mostly grown from seeds sown in pots in a compartment of the plant-quarantine house not used for any other purpose. The culture of *Elsinoe* used (culture 254, A. E. Jenkins) was isolated at Washington, D.C., from cultivated green lima beans collected at Wajay, Cuba, November 5, 1929, by C. Aguiar, chief inspector of the Plant Inspection Service, Department of Agriculture, Commerce, and Labor, Cuba.

[1] Received for publication Aug. 8, 1933; issued January 1934.

[2] JENKINS, A. E. LIMA BEAN SCAB CAUSED BY ELSINOE. Jour. Agr. Research 42: 13-33, illus. 1931.

Most of the inoculations were made by the cotton-plaster method employed by Winston [3] in inoculating *Citrus* with the closely related citrus-scab organism (*Sphaceloma fawcettii* Jenkins). This method consists of spreading small mycelial masses of the fungus grown on potato-dextrose agar on a piece of wet absorbent cotton and then applying this plaster to the uninjured plant surface. The leaf or other part inoculated, together with the cotton plaster, is then wrapped with waxed paper to prevent evaporation. In the present inoculations the paper was held in place by small paper clips, thread, or rubber bands, as was most convenient in each case. The plasters were removed after 24 hours, as preliminary inoculation tests had shown that the duration of the incubation stage did not exceed this period. It was not possible in all cases to arrange the wax paper so that the cotton

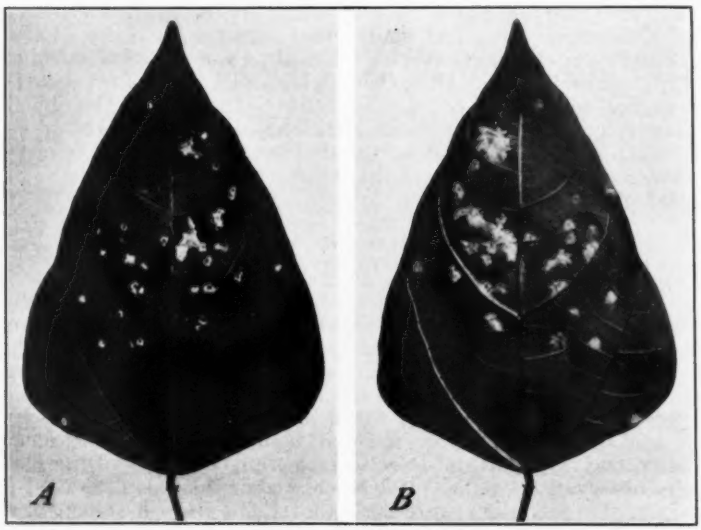

FIGURE 1.—Leaflet of Henderson Bush lima bean 13 days after inoculation on upper surface with a culture of *Elsinoe*: *A*, Upper leaf surface; *B*, lower leaf surface. × 4/5. Santiago de las Vegas, Cuba, Apr. 7, 1930.

would remain moist for 24 hours without at the same time causing injury to the tender growth. Such injury occurred in a number of the inoculation tests that were omitted from table 1.

A few additional inoculations on cultivated lima beans were made by means of a medical atomizer, with which the plants were sprayed with a water infusion of a culture. Since the relative humidity of the quarantine house was somewhat below that prevailing outdoors, these plants dried quickly. Other plants were inoculated by the same method except that they were sprayed first with water alone and, after inoculation, were covered for 24 hours with a large bell jar in order to maintain as nearly as possible a saturated atmosphere during this period.

[3] WINSTON, J. R. CITRUS SCAB: ITS CAUSE AND CONTROL. U.S. Dept. Agr. Bul. 1118, 39 pp., illus. 1923.

In all cases the cultures used as inocula were several weeks old and probably contained few or no conidia at the time they were transferred to the cotton plasters. Conidial formation may have taken place, however, soon after the culture was transferred from the test tube to the wet cotton. Conidial formation under similar conditions has already been described.[4]

INOCULATIONS ON LIMA BEANS

Table 1 gives the results of inoculations that were made on cultivated and on wild or naturalized lima beans by means of the cotton-plaster method. These data show that under suitable conditions the *Elsinoe* causing lima-bean scab readily infects the uninjured leaves and pods of the wild or naturalized lima bean and those of the common horticultural varieties listed. The data also show that infection occurs on tender leaves between 1.5 cm and 5.0 cm in length, the lesions usually becoming visible within 5 to 9 days. Where the upper leaf surface was inoculated the lesions were convex above and concave below; the reverse situation obtained when the lower leaf surface was inoculated. The leaflet shown in figure 1 was inoculated on the upper surface when 2 cm in length; it was photographed 13 days later. Typical scab lesions were obtained on tender pods from 1 to 3.5 cm long in about the same length of time as they were obtained on leaves. On a large, almost mature green pod, flat reddish lesions were produced, but actual infection may not have resulted in this older growth. Noninfection in the case of the tender stems and of a few of the inoculated leaves was possibly due to faulty technic or, as sometimes occurs, to the failure of conidia to form when a culture of the fungus is transferred to a humid environment.

As was to be expected of the plants inoculated by means of the medical atomizer, only those held under bell jars for 24 hours after inoculation became infected. Infection was evident within 7 days on several of the younger leaves, their leaflets then measuring from 4.5 cm to 11 cm in length. The checks remained healthy. The results of this experiment are consistent with observations made at Loiza, P.R., during the winter and spring of 1932.[5] The disease was abundant there until the middle of February. During this period there was a great deal of rain. The disease almost disappeared from the plantings, however, during the 2 months of drought that followed, although it was present in other districts where the drought was less severe. The controls for all these inoculations on lima beans remained healthy.

[4] JENKINS, A. E. (See footnote 2.)
[5] Data obtained from the Bureau of Plant Quarantine, U.S. Department of Agriculture.

TABLE 1.—*Results of inoculations on lima bean by the cotton-plaster method, 1930*

Type or variety	Part inoculated	Length of leaflet or pod (cm)	Date inoculated	Result		Comment
				Positive	Negative	
Bush	Tender leaflet, lower surface.	2	July 15	+	.....	Infection distinct in 8 days.
	Large green pod	.....	Aug. 1	+	.....	Small reddish spots only in 11 days.
	Tender pod	3	Aug. 7	+	.....	Infection visible in 7 days, distinct in 9 days.
	do	2.5	do	+	.....	Typical spots.
	do	3.25	Aug. 12	+	.....	Infection visible in 8 days, distinct in 11 days.
Fordhook Bush	Tender leaflet, lower surface.	3.8	Aug. 26	+	.....	Infection distinct in 8 days.
	Leaflet from same plant as the above one.	2	do	+	.....	Infection faintly visible in 6 days, distinct in 8 days.
Henderson Bush	Tender leaflet, upper surface.	3	Mar. 18	+	.....	Spots distinct in 9 days. Convex above.
	Tender leaflet	1.5	Mar. 20	+	.....	Infection visible in 9 days.
	Tender stem	.....	do	.....	—	
	Tender leaflets	3	do	.....	—	
	do	5	do	.....	—	
	Tender leaflet, upper surface	2	Mar. 25	+	.....	Infection distinct in 7 days.
	Tender leaflet, lower surface.	2.5	do	+	.....	Do.
	Tender pods	1 2	Apr. 9	.....	.....	Both withered and fell off; check normal.
	Flower spike with tender pod.	1	do	+	.....	Typical spots 16 days later on pods.
Challenger Pole	Tender leaflet, upper surface.	3	Mar. 22	+	.....	Spots distinct in 9 days.
	End of tender shoots with unfolding leaves.	.....	do	.....	—	
	Tender leaflet, upper surface.	2.5	Mar. 27	+	.....	Infection visible in 5 days.
	Tender leaflet, lower surface.	3	Aug. 30	+	.....	Infection visible in 6 days, distinct in 8 days.
Frijol caballero (pole type).	Tender leaflets, upper surface.	1.75	Apr. 2	+	.....	
	Tender leaflets	5	do	+	.....	Infection visible in 7 days.
	Tender leaflets, both surfaces.	4	Apr. 9	+	.....	Infection distinct in 9 days.
	Tender leaflet, lower surface.	2.5	July 15	.....	—	
	do	4	Aug. 1	.....	—	
	Tender leaflet	3.5	Aug. 7	+	.....	Infection visible in 6 days, distinct in 7 days.

INOCULATIONS ON OTHER LEGUMES

As previously stated, the cotton-plaster method was employed in all the inoculations on leguminous plants other than lima bean. The results were all negative (table 2). In addition to those reported in table 2, five tests were carefully made on *Canavalia gladiata* in the field on June 25, 1930, and on August 24, 1932, but no infection resulted. The tender leaflets ranged from 1.7 to 4.0 cm in length at the time of inoculation.

The uniformly negative results obtained from these inoculations are of particular interest in the case of *Canavalia*, for they are in accord with field observations made in Puerto Rico [6] and in Cuba. Both species of *Canavalia* listed in table 2 have been under observation for approximately 2 years on the grounds of the Agricultural Experiment Station at Santiago de las Vegas, where *Elsinoe* occurs regularly

[6] JENKINS, A.E. (See footnote 2.)

TABLE 2.—*Inoculations on several species of leguminous plants other than lima bean, 1931*

Species	Part inoculated	Length of leaflet (cm)	Date inoculated[a]
Canavalia ensiformis	Tender leaflet	2.5	Mar. 27
	Tender leaflet, lower surface	3.5	Apr. 2
	Tender leaflet, upper surface	5	Apr. 9
	Tender leaflet and end of shoot	1.5	Apr. 15
	Tender leaflet, lower surface	2	Do.
	do	2	Do.
Canavalia gladiata	Tender leaflet, both surfaces	3.5	Aug. 12
	Tender leaflet, lower surface	3.5	Aug. 1
	Tender leaflet, both surfaces	2	Aug. 7
Dolichos lablab	Tender leaflets	2.2	Aug. 30
Phaseolus vulgaris	Tender leaflet	3.7	Oct. 4
	Tender leaflet, both surfaces	2	Mar. 25
Pisum sativum	do	4	Apr. 9
	Tender leaflet, lower surface	1.5	July 15
Stizolobium deeringianum	do	2.5	Do.
	do	5	Aug. 12
	do	1.5	Aug. 30

[a] Results of all inoculations were negative.

to some extent on lima beans; but no infection has been found. In addition, *C. ensiformis* has been grown simultaneously for 6 months among lima beans during the present season (November 1931 to April 1932) at El Cano, Habana. Actual counts of spotted pods showed that infection on the lima beans by *Elsinoe* increased from a small amount during the first month or two to from 90 to 100 percent by the first of April. No evidence of the disease could be found on *Canavalia*.

The only other member of the genus *Elsinoe* described on a legume is *E. calopogonii* Syd., reported on *Calopogonium caeruleum* in Brazil.[7] On August 9 and 10, 1932, inoculations were also made on this legume growing on the station grounds and showing no infection by *Elsinoe*. Six leaflets ranging from 1.5 to 3.5 cm in diameter were inoculated. These remained free from infection, whereas similar inoculations made at the same time on lima beans in pots in the plant-quarantine house became more severely infected than those referred to earlier in this paper.

It thus appears that the species of *Elsinoe* causing lima-bean scab will not infect either *Canavalia ensiformis* or *C. gladiata*, and that *E. canavaliae* is not distributed in Cuba and Puerto Rico on either species of *Canavalia;* moreover, it is evident that the lima-bean organism is not pathogenic on *Calopogonium caeruleum*, on which *E. calopogonii* has been reported. As previously explained, *E. canavaliae* was originally described on *Canavalia gladiata* in the Orient, and has been reported there on *C. ensiformis;* however, the latter host may have been confused with *C. gladiata*.[8]

TAXONOMIC DISCUSSION

In identifying the *Elsinoe* on lima bean it was pointed out that *Elsinoe* had not been recorded on lima beans in the Orient,[8] although collections of other fungi on this host had been made there. *Elsinoe*

[7] SYDOW, H., and SYDOW, P. FUNGI AMAZONICI A CL. E. ULE LECTI. Ann. Mycol. 14: 65-97, illus, 1916.
[8] JENKINS, A. E. (See footnote 2.)

has since been found on fresh cultivated lima beans from Mexico [9] collected in 1931 and on dried phanerogamic herbarium specimens of both wild and cultivated lima beans from central Mexico [10] that were collected from 1890 to 1925. Its presence on lima beans on the mainland of the Western Hemisphere suggests that it has long occurred here on this native plant.

Through correspondence with botanists and examinations, particularly of phanerogamic herbarium material, the junior writer has made an additional effort to learn whether a species of *Elsinoe* occurs on lima bean in the Orient or on *Canavalia* in tropical or subtropical regions of the Western Hemisphere. Thus far only negative data have been obtained. As already indicated, the lima-bean organism evidently does not infect *Calopogonium caeruleum*, on which the only other named species of *Elsinoe* occurring on a legume has been described.

The data herein presented certainly indicate that the lima-bean fungus is at least physiologically different from *Elsinoe canavaliae* and *E. calopogonii*. So far as known, all three fungi are similar morphologically though of different known distribution. Pending further investigations, particularly of the two described species, when material is available, it is convenient now to classify the lima-bean organism also as a distinct species. If warranted by data gained from additional studies of these organisms, possibly it will be preferable later to treat them as varieties or physiological forms of *E. canavaliae*, the first species of *Elsinoe* to be described on a legume.

TECHNICAL DESCRIPTION

Elsinoe phaseoli Jenkins, n. sp.

Conidial stage, *Sphaceloma*. Conidiophores aggregated in center of young lesions or developed in marginal zones of older lesions, at first hyaline, then dusky or dark colored, unbranched, blunt or pointed, continuous or 1-septate, closely compacted, forming minute acervuli or sporodochia, or more or less effuse, arising from hyaline or colored hyphae passing through the disrupted epidermis parallel to the surface of the leaf, 20μ or less in length by 4μ in width, bearing conidia at their tips; some conidia minute spherical, hyaline, others ovoid or oblong-elliptical, biguttulate or not, hyaline, becoming dusky, reaching 10μ by 4μ; ascomata mostly epidermal, becoming erumpent, often composing small punctiform groups, 140μ, more or less, across; asci ovoid or subellipsoid, becoming obclavate with the rupture of the gelatinous outer wall, unruptured asci up to 30μ by 40μ, eight-spored with spores borne parallel to each other in the ascus; ascospores oblong-elliptical or sausage-shaped, basal part sometimes more obtuse than apical, hyaline, but may be stained red or brownish from coloring matter in matrix, becoming 3-septate, or frequently muriform, by formation of a septum in one of the central cells, 13μ to 15μ by 5μ to 6μ, sometimes breaking apart at median septum, germination by means of sprout conidia; both asci and conidia may swell considerably, asci becoming constricted at cross walls and conidia becoming at least 1-septate and constricted at septum.

Status conidiophorus, *Sphaceloma*. Conidiophoris primum hyalinis, dein fuscidulis vel fuscis, non ramosis, obtusis vel acutis, continuis vel uniseptatis minutos compactos acervulos formantibus, vel effusis, 20$\mu\times$4μ, conidia apice gerentibus; conidiis aliis minutis, globosis, hyalinis, aliis ovoideis vel oblongo-ellipticis, biguttulatis, fuscescentibus, 10$\mu\times$4μ; ascomatibus plerumque subepidermicalibus erumpentibus saepe caespites parvos, punctiformes 140μ diam. formantibus; ascis ovoideis vel subellipsoideis 30$\mu\times$40μ, octosporis; ascosporis parallele dispositis, oblongo-ellipticis vel allantoideis, basim versus obtusis, hyalinis vel ex coloratione matricis brunneolis, 3-septatis, saepe ex septo longitudinali unae cellulae centralis muriformibus, 13μ–15$\mu\times$5μ–6μ, interdum ad septum rumpentibus; ascis et conidiis multo inflatis, ascis septo constrictis, conidiis ad minimum uniseptatis, septo constrictis.

[9] JENKINS, A. E. ADDITIONAL DATA ON THE RANGE AND PREVALENCE OF LIMA-BEAN SCAB. (Phytopathological Note.) Phytopathology 21 : 559. 1931.

[10] JENKINS, A. E. FURTHER STUDIES OF LIMA-BEAN SCAB. Phytopathology 23: 662-666, illus. 1933.

Distribution.—On wild or naturalized and cultivated lima bean (*Phaseolus lunatus macrocarpus* Benth.), Nayarit and Sinaloa, Mexico; El Salvador, Nicaragua, Costa Rica, and Guatemala, Central America; Cuba, Puerto Rico, Dominican Republic, and possibly Jamaica.

Symptoms.—Disease characterized by hyperplastic lesions on leaves, stems, and pods. Leaf lesions circular to irregular, usually raised on upper surface, sometimes confluent, ranging from mere dots to about 5 mm in diameter, frequently visible on both leaf surfaces; commonly vinaceous buff.[11] Stem lesions usually raised, ranging from minute spots to elliptical or elongate areas 1 cm or more in length, where confluent sometimes covering stem for a distance of 2 cm; generally vinaceous buff, sometimes bordered by purplish brown. Pod lesions often swollen, circular to irregular, ranging from mere flecks to lesions 1 cm in diameter; in general, younger lesions brick red to Kaiser brown; somewhat older lesions light cinnamon drab bordered with diamond brown; mature lesions avellaneous, to lighter at center, with reddish to purplish border. Surface of lesions may be black or nearly black owing to growth of *Elsinoe* thereon.

Type specimen.—On cultivated lima bean, Wajay, Havana, November 5, 1929, C. Aguiar. This specimen and others deposited in mycological collections of Bureau of Plant Industry. Culture 254, A. E. Jenkins, isolated from specimen just cited, has been deposited in Centraal Bureau voor Schimmelcultures, Baarn, Netherlands.

SUMMARY

Inoculation tests were made on various legumes in the plant- quarantine house at the Agricultural Experiment Station, Santiago de las Vegas, Cuba, in 1930, with a culture of *Elsinoe* causing scab of lima bean, earlier identified tentatively as *Elsinoe canavaliae* Rac. The infection period was limited to approximately 24 hours. The cotton-plaster method (described) was used and infection readily obtained on leaves and pods of both wild or naturalized and cultivated varieties of lima bean. The cultivated varieties included Fordhook Bush, Henderson Bush, Henderson Pole, and Challenger Pole. Infection occurred on both surfaces of leaflets measuring from 1.5 to 5 cm in length when inoculated, and on tender pods measuring from 1 to 3.25 cm in length when inoculated. In general, the lesions became visible in from 5 to 9 days. Lima-bean plants that were inoculated by being sprayed with water containing bits of a culture became infected in 7 days when covered with bell jars during the infection period, but when not so covered the results were negative.

No infection resulted in inoculations by the cotton-plaster method on *Calopogonium caeruleum*, *Dolichos lablab*, *Phaseolus vulgaris*, *Pisum sativum*, *Canavalia gladiata*, *C. ensiformis*, and *Stizolobium deeringianum*.

The lima-bean *Elsinoe* is now better known than either of the two other species of *Elsinoe* reported on legumes, namely, *E. canavaliae*, occurring on *Canavalia* in the Orient, and *E. calopogonii*, on *Calopogonium* in Brazil. For the present, or until these two described species are studied further, it seems advisable to regard the *Elsinoe* on lima bean as a distinct species. The name *E. phaseoli* Jenkins is therefore proposed for it, and a technical description is given

[11] Color readings are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D.C. 1912.

MODE OF ENTRANCE AND PERIODS IN THE LIFE CYCLE OF CRONARTIUM RIBICOLA ON PINUS MONTICOLA [1]

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INTRODUCTION

The range of the white pine blister rust (*Cronartium ribicola* Fischer) in western North America now extends practically from Alaska to California and from the Pacific coast to Montana. In this greatly enlarged range the rust occurs on new hosts under different climatic and environmental conditions. Investigations of the disease in the West during the last 10 years have resulted in the accumulation of a large volume of field data. The present paper reports the results of studies of the early stages of the rust on native western white pine (*Pinus monticola* Doug.), the chief aecial host in the new range.[3] These stages are of special importance, since during incubation the rust is inconspicuous or unrecognizable and the extent and intensity of infection are therefore in doubt. It is primarily in the early stages that the rust has been widely distributed on shipments of seedling white pine.

MODE OF INFECTION

The mode of infection of the pines by *Cronartium ribicola* was not known until it was demonstrated by the experiments of Clinton (*1*) [4] and Clinton and McCormick (*2*, *3*) in 1917 and 1918. Inoculations by Klebahn (*5*) in 1903 and by Tubeuf (*16*) in 1914 had produced the typical infection spots in the needles, but the connection of these spots with infection in the bark was not understood. Klebahn (*5*) was not sure how the fungus entered the bark. He stated in 1918 (*6*) that although it had been shown that the needles could become infected it had not been shown that the mycelium grew from them into the bark. Tubeuf (*16*) believed that entrance was effected usually by direct infection of the young shoots. McCubbin (*10*, *p. 95*), from field observations, concluded that most of the infection occurred in the bases of the leaf fascicles. The work of Clinton and McCormick (*3*) demonstrated that the germ tubes entered through the stomata of the needles and that the typical mode of infection of the bark was by growth of the hyphae down through the vascular bundles from the infection spots on the needles.

It is still considered possible that the rust may sometimes infect the young bark directly. Spaulding believes that such is the case

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[2] The writer desires to acknowledge the efficient help of J. L. Mielke, W. V. Benedict, C. N. Partington, T. S. Buchanan, J. W. Kimmey, W. F. Cummins, and A. A. McCready, who worked as assistants to the writer during the collection of data basic to this paper.

[3] The studies were made chiefly in southwestern British Columbia, where the disease has been longest established. Because of the diversity of conditions represented in this territory, it is believed that the results secured there, supplemented by preliminary data from the newer sections, will apply fairly closely to the entire present range.

[4] Reference is made by number (italic) to Literature Cited, p. 804.

(*13, pp. 26–27*) but that most infection (*14, p. 594*) occurs in the manner described by Clinton and McCormick. Although unsuccessful in their attempts to infect the bark directly, Clinton and McCormick (*3, p. 441*) believe that direct infection may occur to a slight extent in bark that is young enough to have stomata through which the germ tubes of the sporidia may enter.

Field evidence in the present studies supports the conclusions of Clinton and McCormick (*3*) and indicates that the mode of infection in *Pinus monticola*, as in other aecial hosts of *Cronartium ribicola*, is through the needles. After favorable infection years, the golden yellow to brownish spots that mark the location where infection took place in the needle are generally abundant wherever much infection of pines is in progress. The outstanding field evidence observed by the writer showing that the bark is typically infected through the needles in *P. monticola*, however, is as follows: The first characteristic yellow to orange discoloration or swelling in the bark, which marks the appearance of the incipient canker, almost invariably develops symmetrically around a single needle bundle. Aside from its central location in the discolored area this bundle is generally distinguished by a marked swelling of its base. An examination of the needles of this bundle will usually disclose at least one definite needle infection spot. Other needle bundles may occur on the discolored area, but, except where there is evidence that the rust has entered from more than one needle bundle, their bases are generally not swollen. Frequently the central bundle, through which infection entered, persists considerably longer than the rest. Where all needles have fallen the enlarged scar of this bundle is generally conspicuous, or at least is recognizable, in the center of the discoloration.

Indirect evidence that the mode of infection is through the needles is found in the distribution of the incipient cankers resulting from any given year's infection on the various years' internodes exposed to the infection (*7, 8*). If the internodes formed the year of infection are designated as *a*, those of the preceding year as *b*, those 2 years older as *c*, etc., the occurrence of needles at the normal time of infection in late summer and early fall is limited primarily to *a*, *b*, *c*, and *d*, although *e* will have some, *f* may have a few, and *g* or even *h* may rarely have a few. The occurrence of the incipient cankers is confined to internodes within these age limits, i.e., to growths upon which needles may occur at the time of infection, and an average of over 99 percent of the cankers develops on *a* to *d* where the chances are best for entrance through the needles. Based on tabulations of approximately 6,000 cankers from study areas throughout the rust's western range, the average distribution of the incipient cankers on the pines is as follows: *a*, 10− percent; *b*, 53+ percent; *c*, 31+ percent; *d*, 5+ percent; *e*, 0.5 percent; *f*, trace; and *g* and older internodes, 0 percent.

The sparsity of the cankers on *e* and older internodes corresponds with the sparsity of needles on these internodes. Most of the needles on *d* are cast at the end of the infection season, so that relatively little time is available for the entrance of infection through these needles. As might be expected, therefore, if infection enters through the needles, *d* has only a relatively small proportion of the cankers. *c* loses part of its needles at the end of the infection season and most of the remainder 1 year later. The chances for the entrance of in-

fection through the needles on *c* are therefore relatively good. The proportion of the cankers on this growth averages 31 percent. *b* holds its needles a full year longer than *c* and has 53 percent of the cankers. The needles on *a* are held a full year longer than those on *b*; in other words, all of them are normally held 3 full growing seasons, including the season of infection, and the majority are held 4 seasons. The cause of the small proportion of cankers on *a* appears to be that its needles, i.e., the current season's needles, are resistant to infection (*8*).

Those who have stated or suggested that infection enters the bark directly rather than through the needles, or that direct infection of the bark occurs at all, have generally agreed that it will occur primarily on the new shoots where stomata are present and where the tender young bark permits the entrance of the germ tubes of the delicate sporidia. On this basis *a* should have most of the cankers; actually it has only about 10 percent as compared with 53 percent on *b*, 31 percent on *c*, and 5 percent on *d*. The only explanation for this is that the mode of infection is through the needles.

INCUBATION PERIOD

DEFINITION

The term "incubation period" in its ordinary application refers to the time between infection and the appearance of disease. Since *Cronartium ribicola* infects through the needles and the yellowish or brownish spots on the needles represent the first symptoms of the disease, the incubation period might strictly be defined as the time from infection to the appearance of these spots. But there is considerable variation in the development and character of the spots. In examining *Pinus monticola* under natural conditions it is generally possible to be reasonably certain of the identity of the spots after they have become enlarged and have assumed the characteristic golden-yellow color. However, before this stage is reached and afterward when the infected needle tissues begin to die and turn brown, or where the development is atypical, there is frequently no definite macroscopic means of distinguishing the blister rust from spots resulting from other causes. Consequently, even when no other spots are present, there is always a considerable proportion of the blister-rust spots that cannot be identified with certainty. Furthermore, where, as must often be the case, the fungus fails to reach the bark before the needle is shed, the infection in the needle is of no practical significance. In exceptional cases, moreover, it is possible that infection takes place directly in the young bark (*3, p. 441; 13, pp. 26–27*). For these reasons, the time required for the development of the needle spot is an unreliable and unsatisfactory measure of the incubation period of blister rust, at least on native *P. monticola*.

The next stage in the development of the fungus is characterized by discoloration and swelling of the bark. After varying periods these symptoms are followed by the production of pycnia and later of aecia.

In conformity with the most generally accepted practice in the treatment of the early stages of the aecial forms of *Cronartium*, Spaulding and earlier writers (*13, pp. 24–26*) have considered the incubation period of *C. ribicola* on its aecial hosts as the period from infection to the production of aecia. Such a definition is of course necessary and agrees exactly with the meaning of the term when, as

is frequently the case with other species of the genus, the disease cannot be recognized earlier. The symptoms of infection by *C. ribicola*, however, are usually apparent on most of its pine hosts considerably before this time. In *Pinus monticola* the conspicuous yellow-to-orange discoloration in the normally smooth green bark readily establishes the identity of the incipient canker at a very early stage and generally before the formation of pycnia. Since the time elapsing between the appearance of the incipient canker and the production of aecia is highly variable, to define the incubation period as extending from infection to the production of aecia would not only make the period unduly long, variable, and indefinite, but would complicate the subject unnecessarily. The incubation period for *P. monticola* is therefore defined as the period from infection to the appearance of discoloration or swelling of the bark sufficient for a ready and accurate macroscopic diagnosis.

LENGTH OF THE PERIOD IN TREES BEYOND THE SEEDLING STAGES

In the natural infection areas of the western range of the rust, the trees vary in size from seedlings to veterans. Generally the best areas for study have been located in young reproduction ranging up to 30 or 40 feet in height, although trees of all sizes have been included. On the youngest seedlings, as will later be shown, the incubation period of the rust is shorter than on trees more than about 3 feet high or 8 years of age. Since most of the foliage is on the larger trees, however, it is obvious that most of the infection will be found on trees beyond the height and age limits just specified. On trees beyond these limits the writer has never seen an incipient canker on internodes younger than those formed two seasons before the season of examination. Minute orange discolorations have occasionally been observed about the bases of needle bundles in bark formed the year preceding that of examination, but it was impossible to determine from macroscopical examination whether they were young infections or whether they were caused by aphids, which often induce a similar type of discoloration. Since the symptoms were uncertain and the discolorations could not be definitely classified, these cases were excluded from the calculations of the length of the incubation period on the older trees.

Throughout the present range of the rust on *Pinus monticola* the first teliospores usually begin to form on *Ribes* during June. Their production reaches maximum proportions in late July, August, or September, depending on the weather. By the end of November *Ribes* plants are generally defoliated and the teliospores have germinated. Late rains saturate the surface duff and assure germination of any teliospores that may remain on the fallen leaves. Consequently the amount of infection that results from fallen leaves some time after general defoliation, that is, after about December 1, is negligible, and for practical purposes may be disregarded. Infection of pines, therefore, may occur from about June to November, inclusive, and is generally heaviest in August, September, or the first part of October, after most of the teliospores have formed.

The earliest formation of incipient cankers recorded in general (i.e., on trees more than about 3 feet in height or 8 years of age) has been in April of the second year following that of infection. Thus the minimum incubation period in general for *Pinus monticola* is at least 16 months, or from infection at the end of November of one year

to April of the second succeeding year (fig. 1, *A*). Since infection usually occurs several months before the end of November, the incubation period is probably nearer 18 months.[5]

Observations of waves of canker formation resulting from single years of infection at various localities that represent a wide diversity of conditions in southwestern British Columbia and also include areas from the newer sections have shown that most of the incipient cankers appeared between May and November of the second year after the year of infection. Since most of the infection occurs between late

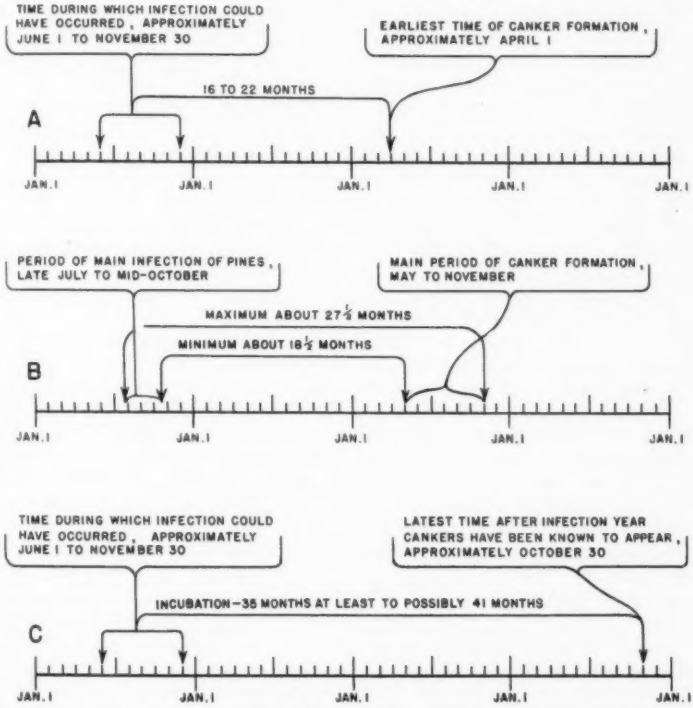
TIME DURING WHICH INFECTION COULD HAVE OCCURRED, APPROXIMATELY JUNE 1 TO NOVEMBER 30
EARLIEST TIME OF CANKER FORMATION, APPROXIMATELY APRIL 1
16 TO 22 MONTHS
A
JAN. 1
JAN. 1
JAN. 1
JAN. 1
JAN. 1
PERIOD OF MAIN INFECTION OF PINES, LATE JULY TO MID-OCTOBER
MAIN PERIOD OF CANKER FORMATION, MAY TO NOVEMBER
MAXIMUM ABOUT 27½ MONTHS
MINIMUM ABOUT 18½ MONTHS
B
JAN. 1
JAN. 1
JAN. 1
JAN. 1
JAN. 1
TIME DURING WHICH INFECTION COULD HAVE OCCURRED, APPROXIMATELY JUNE 1 TO NOVEMBER 30
LATEST TIME AFTER INFECTION YEAR CANKERS HAVE BEEN KNOWN TO APPEAR, APPROXIMATELY OCTOBER 30
INCUBATION—35 MONTHS AT LEAST TO POSSIBLY 41 MONTHS
C
JAN. 1
JAN. 1
JAN. 1
JAN. 1
JAN. 1

FIGURE 1—Incubation period of *Cronartium ribicola* on *Pinus monticola*: *A*, Minimum incubation period (see footnote 5); *B*, maximum range of usual incubation period; *C*, maximum incubation period.

July and mid-October, the usual incubation period is seen to range at most from 18½ to 27½ months (fig. 1, *B*).

Specific studies of individual infection waves have shown that for most of the cankers the incubation period averaged from about 21 to 24 months. At Revelstoke, British Columbia, in 1922, for example, sporidia production and infection were concentrated in September, and the resultant canker formation occurred in June, July, and August of 1924, a period of about 20 to 23 months.

[5] E. L. Joy, of the Division of Blister Rust Control, reports finding recently on the larger trees in infected areas in Idaho definite incipient cankers as early as September 15 of the year following the infection year. This would reduce the minimum incubation period for most trees to between 9½ and 15½ months, probably to about 12 to 13 months.

On an experimental plot at Apex, British Columbia, the infection was transmitted from two well-isolated and artificially infected bushes of *Ribes viscosissimum* Pursh in 1928. Detailed records of the development of infection on the *Ribes* bushes and of weather conditions showed that the main sporidia production and most favorable conditions for infection of the pines occurred in September. Between May 1930 and about August 1931, 203 incipient cankers developed. Most of these were produced in July, August, and September 1930, the incubation period being between about 21 and 24 months for the majority of the cankers.

At Thurston Bay, British Columbia, in 1922, observations of *Ribes* infection and of weather conditions showed that the main infection of pines must have occurred in October. The resultant canker formation was concentrated in September and October 1924 after a lapse of about 22 to 24 months. On a similar basis, the incubation period was calculated at between 21 and 22 months after infection at Thurston Bay in 1924; between 20 and 22 months after infection at Qualicum Beach, British Columbia, in 1924; between 21 and 23 months after infection at Qualicum Beach in 1927; between 22 and 26 months after infection at Daisy Lake, British Columbia, in 1923; and between 23 and 25 months after infection at Owl Creek, British Columbia, in 1924.

Frequently a considerable proportion of the cankers resulting from a given year's infection make their appearance in the third growing season after that of the infection year. For example, about 10 percent of the incipient cankers resulting from the 1921 infection at both Chee Kye and Daisy Lake, British Columbia, did not form until April 1924. At Revelstoke about 25 percent of the cankers resulting from the 1922 wave of infection did not appear until the growing season of 1925 and some of them were still forming in August of that year. The incubation period for these cankers was 33 months at the minimum and 35 months if counted from September 1922, when conditions were most favorable for infection. Similarly, at another infection area several miles north of Revelstoke, at least 20 percent of the cankers of 1927 origin did not appear until the growing season of 1930, some of them forming as late as October. The incubation period for these cankers was not less than 35 months and was probably closer to 38 months. Assuming that some of the infection occurred in June 1927 and that some of the cankers appearing in October were the result of this infection, the incubation period for such cankers would be 41 months (fig. 1, *C*).

What the maximum incubation period may be in individual cases is not known. Incipient cankers are occasionally found forming on internodes as old as 9 years, which would make a possible maximum of 9 years in incubation. However, since infection occurs through the needles and *Pinus monticola* holds some of its needles occasionally as long as 8 seasons, it is far more likely that such cankers have entered these internodes through persistent needles long after the year in which the internodes originated. It is improbable that the incubation period is ever more than 4 years. As already shown, the incubation period for most of the cankers is between 20 and 26 months and ranges for decreasing numbers of cankers to about 38 or 39 months, with a possible maximum of about 41 months.

It should be borne in mind that in nature the growth of the fungus is very much retarded during the colder season. Measurements of cankers (not reported here) indicate that growth during the months intervening between growing seasons—or, roughly, from October to March, inclusive—is but from a fifth to a sixth of the growth made between April and September, inclusive. In other words, growth is practically as great during a single month of the growing season as during the entire 6 months of late fall, winter, and early spring. Variation in the month of infection, therefore, will act disproportionately upon the length of the incubation period, generally tending to shorten the period if infection occurs early and lengthen it if infection occurs late.

Thus, if infection occurs at the end of June, the individual infection will have the following months to develop to the point of canker incipiency: the 3 active months of July, August, and September; the following 6 inactive months, equivalent to 1 active month; 6 active months; 6 more inactive months; and 6 more active months—a total of 27 months, active and inactive, or the equivalent of 17 active months, from the time of infection to the end of the second season following that of infection. If infection occurs at the end of July, the equivalent of 17 active months would extend the time to the beginning of the third growing season following that of infection, making a total of 32 months, active and inactive. However, if only 16 active months are required for incubation, infection at the end of July would permit the formation of the incipient canker at the end of the second season following that of infection after a total of only 26 months.

Recapitulating the former conclusions as to the general length of the period required for incubation by calculating the 6 months from October to March as the equivalent of 1 active month and each month from April to September as 1 active month, the period would range from a possible minimum of about 8 [6] to a possible maximum of 25 active months, and average between about 10 and 16 active months.

Undoubtedly it is not strictly accurate to give equal weight to each of the active months from April to September. Probably in May, June, and July, or in June, July, and August, growth activity of the rust is somewhat greater than in the other months of this period. This point has yet to be fully studied. Undoubtedly such differences will vary with the weather and locality and will increase with altitude and northward latitude. Obviously these differences will accentuate the disproportionate effect of variation in time of infection on length of incubation period.

At the upper elevations for *Pinus monticola*, where the growing season may be as much as 2 months shorter than in the main range of the species, the active season for the rust should be calculated at 5, or even 4, instead of 6 months, depending on local conditions, and the remainder of the year as the equivalent of 1 active month. It is to be expected that reduction of the active season for the rust will be reflected in a longer period for incubation. This tendency has been very noticeable in the study areas of the highest elevation and shortest growing seasons from which data are available; in these areas the appearance of considerable proportions of the cankers in the third

[6] In the case of the incipient cankers reported by E. L. Joy (see footnote, p. —), the lowest possible minimum would be about 6½ active months, or from Nov. 30 of one year to Sept. 15 of the next.

season after infection is a frequent occurrence, whereas in the study areas, at about sea level, where the growing season has been longest, it is a rare occurrence.

In this connection it should be stated that all these higher areas are situated much below the upper altitudinal limits for *Pinus monticola*. The data on incubation at or about these limits is too fragmentary to form a basis for conclusions. It is possible that at these extremes a large majority of the cankers may not appear until the third season after infection.

Of the areas studied those of relatively higher elevations and shorter growing seasons more closely resemble the commercial range of *Pinus monticola* in general climatic conditions than do the sea-level areas. It is interesting to note, therefore, that preliminary data from the commercial range in Idaho also indicate a frequent and considerable extension of canker formation into the third season after infection.

Another factor which might influence the incubation period is the difference in growth rate of the fungus induced by variations in the thickness and thrift of the bark. The growth of the canker is strongly favored by the thickness, and, to a large degree, by the vigor of the infected bark, and consequently is more rapid in the larger, thriftier branches and stems than in the smaller, slower-growing twigs and branches. It might, therefore, be expected that the incubation period would be shorter and that the incipient cankers would appear earlier on the larger and thriftier branches. H. N. Putnam, of the Division of Blister Rust Control, informs the writer that he has observed evidence of such a relationship in a number of areas. The writer has not been able to establish definite evidence of it in his own work, but he has observed a noticeable difference in the size of the initial discolorations, which in general depends on the size and thrift of the part affected.

LENGTH OF THE PERIOD IN SEEDLINGS

On very young trees, ranging up to 4 or 5 years of age, incubation generally requires a full year less than on the older trees. This was first observed by the writer in 1924 in an experimental planting of five hundred 3-year-old nursery stock of *Pinus monticola* that had been made at Daisy Lake, British Columbia, in 1923, by the Dominion Department of Agriculture.[7] Since then the same phenomenon has been noted as typical of the development of the rust on trees of this age class.

The trees of the experimental planting just mentioned were healthy transplants from the United States Forest Service nursery at Wind River, Wash. No instance of white pine blister rust was then known at the nursery or in the vicinity of it; it is certain that the trees were not exposed to infection there, for western white pines remaining at the nursery did not develop infection.

The plot in which the trees were set out at Daisy Lake was in the center of an infection area close to heavily infected *Ribes*. Planting was done on April 10, 1923. Between mid-June and defoliation of *Ribes* in the fall, the trees were subjected to heavy infection. Data on weather and on the production of sporidia indicated that the main infection of the pines occurred in August.

[7] DAVIDSON, A. T. RESULTS OF PAST SEASON'S WORK, PRESENT SITUATION, AND FUTURE PLANS. Report of Proceedings, Meeting of Trustees, Western White Pine Blister Rust Conference, held in Spokane, Wash., Jan. 7 and 8, 1924, pp. 7–18. [Mimeographed.]

On June 18, 1924, incipient cankers resulting from this infection had begun to appear. From that time until the end of the growing season, 265 of these cankers had formed. It was evident from the rate at which the cankers grew that the first of them must have appeared about 2 weeks before the first examination of the trees on June 18. The incubation period for these first-formed cankers was not more than 11½ months, i.e., from mid-June in 1923, when the first teliospores were produced, to the 1st of June 1924, when the cankers made their appearance, or the equivalent of less than 7 active months. Assuming that the infection occurred in November, the incubation period may have been as short as 6 months, the equivalent of only 3 active months. This must represent close to the minimum limit for the incubation period of the rust under any conditions. In the present case, since the main infection occurred in August, the minimum period of incubation was probably nearer 9 months, or the equivalent of 4 active months.

Most of the cankers appeared in August or September. Since the majority of these unquestionably resulted from the main infection in August of the preceding year, the incubation period for the majority of the cankers ranged from 12 to 13 months, or from 7 to 8 active months.

For the cankers which made their appearance at the end of the season, the possible range of the incubation period was from 11 to 17 months. Estimated from the time of the main infection, the incubation period was about 14 months, or the equivalent of about 8 active months.

The degree to which the incubation period of the infections of 1923 extended beyond these limits could not be exactly ascertained. When the pines were examined again in November 1925 it was found that many new cankers had appeared that year, almost equaling in number those formed in 1924. But it was evident from the proportion of the cankers found on the 1924 internodes that much additional infection had occurred in 1924, and it was impossible to determine exactly what proportion of these new cankers were of 1923 origin and what proportion were of 1924 origin. Apparently, judging from their relative distribution on internodes of different years and from their stage of development, about one third to one half of them were of 1923 origin. Those falling in this latter category were generally more advanced in development than the rest and had evidently appeared some time earlier in the season.

There was no evidence to indicate that any of the more recently formed cankers were of 1923 origin. It is probable, however, that a few of them were, and that incipient canker formation on these youngest classes of trees may extend to well toward the end of the second season after infection, just as in the older classes of trees it sometimes extends to the end of the third season after infection. This would bring the possible range in the period on the young classes of trees up to a possible maximum of about 30 months. There is no evidence, however, to show that the period extends to any appreciable degree into the third season following infection, and incubation may be considered in general to require at least a year less than on the older trees.

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TIME REQUIRED FOR PRODUCTION OF PYCNIA

Observations during the entire period of study indicate that the formation of pycnia on *Pinus monticola* is strongly influenced by temperature. Depending on local and seasonal climatic conditions, it may begin as early as May and continue as late as November. In warmer localities near sea level it usually begins in June, is heaviest in July and August, and subsides when the weather turns cooler, usually in September; but periods of warm or hot weather may induce a considerable resumption of activity in the production of this spore form in October or even November. At higher elevations the season of production generally begins later by several weeks or more and ends earlier by a corresponding period.

If the incipient canker forms before the middle of the period during which pycnia normally appear, pycnia are almost invariably produced upon it the same year. If it forms during the period of maximum pycnial activity, it may bear pycnia almost immediately. If it forms later in the season, pycnia are generally not produced upon it until the following summer. The period required for production of pycnia after the formation of the incipient canker, therefore, may vary from less than a month to as high as 10 months, depending on seasonal conditions and the time the canker forms. Consequently the period from infection to the production of pycnia is, roughly, from 1 to 10 months longer than the incubation period. The equivalent increase in active months would be 1 to 5 of such months, the maximum being calculated as from the first of September to the first of July of the following year.

TIME REQUIRED FOR PRODUCTION OF AECIOSPORES

In the western range of the rust, aeciospore production on *Pinus monticola* varies widely with seasonal conditions and locality. At low elevations it may begin as early as the last of February, but usually it does not begin until the latter part of March or the first part of April. At high elevations near the upper limits of occurrence of the pine, it may not begin until as late as July, but apparently it is usually under way by the first of June. As stated earlier, however, relatively few data are available from these marginal regions. The heaviest production through the main range of *Pinus monticola* in British Columbia occurs between early April and mid-June. Fairly heavy production has been observed in a few cases, however, to extend well into July or even into August. At high elevations the main production appears to occur usually between mid-May and mid-July and to be concentrated within the relatively short period of a month or less. At lower elevations, also, the period of heaviest production is often short.

Rather frequently at the lower elevations a sporadic and light resumption of aeciospore production may occur in late fall. This has been noted at a considerable number of infection localities at such elevations in several different years in October or November. It usually occurs under the influence of a period of warm and open fall weather in years having an early spring. It has not been noted at high elevations. Practically, it is of little importance, since the aecia are small and dwarfed and occur on only a fraction of 1 percent of the cankers at a time when *Ribes* defoliation is generally in progress

and the spores can cause little or no infection. Nevertheless, it prolongs the period in which aecia may be borne to practically the entire growing season and suggests the possibility of the occurrence of incipient canker formation, pycnial sporulation, and aeciospore production all in the same year. No instance of this sort has been actually recorded, however.

On the majority of the cankers aecia are usually produced in the spring of the year following that in which the cankers first bore pycnia. Frequently, however, a large proportion, sometimes a majority, of the cankers do not produce aecia until the second year after that in which pycnia are produced. Observations and tabulations of cankers of various infection waves have shown percentages bearing or failing to bear aecia in the year following that in which pycnia were first produced approximately as given in table 1.

TABLE 1.—*Aecial production in Cronartium ribicola cankers in various western study areas*

[Cankers had borne first pycnia in year preceding that of observation]

Area of infection wave	Year of observation	Cankers observed		
		Total	Bearing aecia	Not bearing aecia
British Columbia:		*Number*	*Percent*	*Percent*
Thurston Bay	1923	77	51	49
Do	1924	281	55	45
Pender Harbor	1924	134	24	76
Chee Kye	1924	377	89	11
Daisy Lake	1924	143	68	32
Mile 72, Pacific Great Eastern Railway	1924	476	79	21
Revelstoke	1925	184	78	22
Qualicum Beach	1926	65	14	68
Owl Creek	1926	228	36	64
Beaton	1926	171	83	17
Arrow Park	1929	65	51	49
Do	1930	129	64	36
Columbia River plot	1930	1,653	51	49
Eagle Creek plot, Oregon [a]	1930	177	52	48
Average			57	43

[a] Data collected by the Spokane branch of the Division of Blister Rust Control, Bureau of Plant Industry, U.S. Department of Agriculture.

Cankers which fail to produce aecia in the year following that of pycnial production usually produce pycnia again the second summer and aecia the next spring. Sometimes, however, some of the cankers do not produce aecia until the third or even the fourth year after that in which they first produced pycnia. Frequently this is caused by the presence on the cankers of secondary parasites which kill the bark on which pycnia were produced before aecia could be formed. However, the same thing has been observed in cankers that were not visibly affected in any way by secondary parasites.

The cause of this hold over in the development of aecia where the bark remains alive is unknown. Craigie (*4*), working with other heteroecious rusts, has shown that the pycnial stage constitutes an essential sexual step in the life cycle of these rusts. Association of the positive and negative pycniospores in the pycnia is a necessary preliminary to the formation of aecia. The two types of pycniospores are produced in separate pycnia which are usually intermingled on

the same host substratum so that the pycnial fluid containing the spores is easily carried from one pycnium to another by insects, rain, gravity, or other agencies. Any factors that prevent this process generally inhibit the formation of aecia.

Though not yet demonstrated experimentally, the function of the pycnia in *Cronartium ribicola* is doubtless the same as that in the other heteroecious rusts.[8] If this is true, weather and other physical factors preventing a satisfactory production or distribution of the pycnial fluids and spores will probably be found to be chiefly responsible for the delays in aecial production on cankers failing to produce aecia in the season following that of the formation of pycnia. Parasites upon the pycnia, however, may also play an important part.

In this latter connection it is worthy of mention that *Tuberculina maxima* Rostr., a common parasite on the aecia in Europe (*9*, *15*), is also widely distributed and evidently indigenous on the rust in the Pacific Northwest. In certain wet springs this fungus has been abundant at several infection areas here on the aecia, aeciospores, and also on the spots where pycnia had formed the preceding year. It has also been noted abundantly on the pycnia during summer. Although where infection of aecia is heavy aeciospore production and dissemination are considerably reduced, the fungus may actually be of more importance in the reduction of aecia through parasitism of the pycnia. In cases where parasitism of pycnia is extensive it undoubtedly is an important factor in restricting the production of aecia the following spring. In general, however, the occurrence of the fungus in the Pacific Northwest is sporadic.[9]

Cankers affected by secondary organisms may not produce aecia at all. In such cases, however, the canker generally dies within a few years of its formation. Occasionally, the outer portions may die while the rust spreads on down the branch from the lower portions and the secondary organism follows but does not completely overcome the infection. Ultimately, the rust may reach the bole without yet having produced aecia. Here it may produce aeciospores, perhaps 10 or 15 years after pycnia were first produced on the branch. If such an infection enters where the bole is large and the bark is thick and rough, the production of aeciospores may be still further delayed, for such bark greatly retards aecial sporulation and may inhibit it altogether. Infection may also occasionally enter a large trunk directly through a short shoot, killing the shoot before aecia can be produced. In such cases the fungus may vegetate in the thick bark for a period of 5 or 10 years and not produce aeciospores until it has spread from the bole into the thinner bark of some branch.

The period required for the development of aeciospores after the first production of pycniospores is therefore seen to range from a possible minimum of less than 4 months (November to March) up to a possible maximum of perhaps 2 decades or more if secondary-parasite infection is considered, or to over a decade if only the infection through short shoots is considered. In general, it ranges from about 6 months to 2½ years. These periods added to the period required for the production of pycnia make the period from infection to the

[8] A preliminary report of experimental results with *C. ribicola* on *Pinus monticola* by Pierson (*12*) indicates that such is the case. Pycnia fertilized by interchange of pycniospores gave apparent fusions of pycniospores with the filamentous hyphae in 11 cases, whereas unfertilized pycnia gave none.

[9] These relationships have been recently brought out by Mielke (*11*) in a report of this parasite's western distribution and behavior.

production of aecia an indeterminate one of about 2 to 7 years for trees more than about 3 feet high and 8 years old. In special cases the minimum period on such trees may be a few months less and the maximum 10 to 20 years longer.

A somewhat different method must be used to calculate the usual period from infection to the production of aecia. As stated earlier, a large majority of the incipient cankers make their appearance in the second year after the infection year, and the remainder form in the third year after the infection year. Extensive tabulations indicate that the average incidence of cankers is about 85 percent in the second year and 15 percent in the third year after the year of infection.

Of the cankers that appear in the second year after infection, the percentage producing pycnia varies according to the time of year at which the cankers are formed. The tabulations indicate that the average is about 70 percent. Of the cankers that appear in the third year after infection, an average of about 90 percent produce pycnia. Practically 100 percent of the cankers that form too late in the season to bear pycnia that year, produce pycnia the next year. Of the cankers that produce pycnia for the first time in a given year, an average of about 60 percent produce aecia the following spring, and at least 90 percent of the remainder produce aecia the next spring following.

As calculated from these percentages, the cankers resulting from a given infection year produce aecia for the first time after infection in about the following proportions: 36 percent in the third spring after the year of infection, 43 percent in the fourth spring, 18 percent in the fifth spring, and the remaining 3 percent mainly in the sixth and to some extent in the seventh spring.

As stated earlier, most of the production of sporidia and infection of pines occurs between late July and mid-October. The production of aeciospores begins usually between April 1 and mid-May. Therefore, for the 36 percent of the cankers which first produce aecia in the third spring after the year of infection, the usual period from infection to aeciospore production is approximately 2½ to 3 years; for the 43 percent which first produce aecia in the fourth spring, the period required for aeciospore production is approximately 3½ to 4 years; for the 18 percent which first produce aecia in the fifth season, the period required is between 4½ and 5 years; and for most of the remaining small percentage, the period is between 5½ and 6 years.

On trees less than 5 years old, the minimum period from infection to production of aecia is about a year less than on the older trees, but the possible range of the period will be very much less because of the small size of the parts and the thinness of the bark of the young trees. The maximum period on such stock is about 5 years. The usual periods are 1½ to 2 years; 2½ to 3 years; and, to a lesser degree, 3½ to 4 years for cankers producing their first aecia in the second, third, and fourth spring, respectively, after infection.

SUMMARY

Field evidence indicates that in *Pinus monticola*, as in other white pine species, *Cronartium ribicola* gains entrance to the bark through the needles. Typical needle-infection spots are developed. The initial discoloration, which marks the incipient canker in the bark, is

almost invariably centralized symmetrically about a single needle bundle the base of which is usually swollen and the needles of which have at least one characteristic rust-infection spot. Except for the internodes of the infection season, upon which the needles appear to be resistant, most of the cankers form upon the younger internodes where the needles are most numerous and are retained longest.

The incubation period is defined as the period from infection to the appearance of discoloration or swelling in the bark sufficient for an accurate macroscopic diagnosis. On trees more than 3 feet high and 8 years old, the period for most of the infection ranges from about 20 to 26 months, or the equivalent of 10 to 16 active months, if each month of the growing season, from April to September, inclusive, is considered as 1 active month and the intervening inactive season, from October to March, inclusive, is considered as the equivalent of 1 active month. After infection on such trees, the minimum period required for cankers to form is generally not less than 16 months, or the equivalent of about 8 active months. Recent data collected in Idaho by the Division of Blister Rust Control indicate that in certain cases the extreme minimum might have been as little as 9½ months, equivalent to 6½ active months. The maximum period for the latest-formed cankers observed could not have been less than 35 months and might have been as much as 41 months, the equivalent of 21 to 25 active months. It is not probable that the incubation period ever extends beyond 4 years. It varies considerably, depending on the time of infection and seasonal conditions, and is longer at higher elevations, where the season is shorter, than at lower elevations, where growth activity is greater and extends over a longer period.

On the youngest trees, up to 4 or 5 years of age, the incubation period is about a year shorter than on the older trees. Incipient cankers have been observed to form upon them after a possible minimum of 6 months, or an equivalent of only 3 active months.

Following incubation, the period required for the production of pycniospores ranges from less than a month to 10 months.

Following the production of pycniospores, the period required for the production of aeciospores is usually from 6 months to 2½ years. It sometimes extends over 3 or even 4 years, and on the largest trees under certain circumstances occasionally ranges as high as 10 to 20 years. On the youngest trees it rarely extends beyond 2½ years.

On trees more than 3 feet high and 8 years old, therefore, the total time from infection to production of aecia ranges in general from about 2 to 7 years and may occasionally exceed 20 years. The usual period is between 2½ and 5 years. On trees less than 5 years of age the total period ranges from about 1 to 5 years, and is usually from 1½ to 4 years.

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INCREASE IN GROWTH OF LOBLOLLY PINES LEFT AFTER PARTIAL CUTTING[1]

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INTRODUCTION

Information on the growth of trees left after partial cutting, and on the factors affecting it, is of value in planning the management of forest stands. Such information makes it possible not only to predict the growth of trees that have been left by partial cutting but also to select as trees to be left in partial cutting those more capable of benefiting by release. Together with information on other silvicultural characteristics of a species and on economic factors, it forms the basis for applied silviculture. This paper deals with the results of a study of increase in growth at breast height[3] of loblolly pine (*Pinus taeda*) trees left after partial cutting.

REVIEW OF LITERATURE

Many workers have presented data on the increase in growth rate of forest trees after release. Marshall (*11*),[4] studying the growth of hemlock (*Tsuga canadensis*), found that on an average the trees grew five and one half times as fast in diameter at stump height after release as before. Lutz (*9*) and Merrill and Hawley (*12*) found that released hemlock trees showed an increase of from 250 to 350 percent in rate of diameter growth at breast height.

Ponderosa pines (*Pinus ponderosa*) studied by Krauch (*8*) showed marked increase in diameter growth after they were released by removal of part of the stand. In this instance the percentage increase in diameter growth was found to be inversely proportional to diameter at time of release. Dunning (*5*), also working with ponderosa pine, found trees with large crowns more responsive to release than small-crowned trees.

Bentley and Recknagel (*3*) pointed out that red spruce (*Picea rubra*) increased in rate of growth following release. The same tendency was noted for balsam fir (*Abies balsamea*) by McCarthy (*10*), who found that the small trees showed greater increase in diameter growth than the large ones.

Longleaf pines (*Pinus palustris*) studied by Barrett (*1*) showed a marked increase in growth after release. Trees that were small at the time of release showed greater increase in rate of volume growth than the larger trees.

[1] Received for publication Aug. 17, 1933; issued January 1934.

[2] The field work of this investigation was initiated by C. F. Korstian, formerly of the Forest Service, to whom the writer is indebted for later assistance. Acknowledgment is also due F. X. Schumacher and D. Bruce, of the Forest Service, for valuable suggestions in the statistical analysis, and Mary L. Denoyer, of the Forest Service, for assistance in the computational work.

[3] All diameter and basal-area data mentioned in this paper represent measurements taken at breast height (4.5 feet above ground) outside bark, unless otherwise specified.

[4] Reference is made by number (italic) to Literature Cited, p. 821.

Chapman (*4*), investigating the growth of several loblolly pines after partial cutting, found a marked increase in rate of diameter growth. He concluded that the rate of increase depended upon the relative length of the crown at the time of release.

CHARACTER OF DATA

The data used in this study were taken during 1927 and 1928 in nine loblolly pine stands on the Atlantic coastal plain of Virginia and

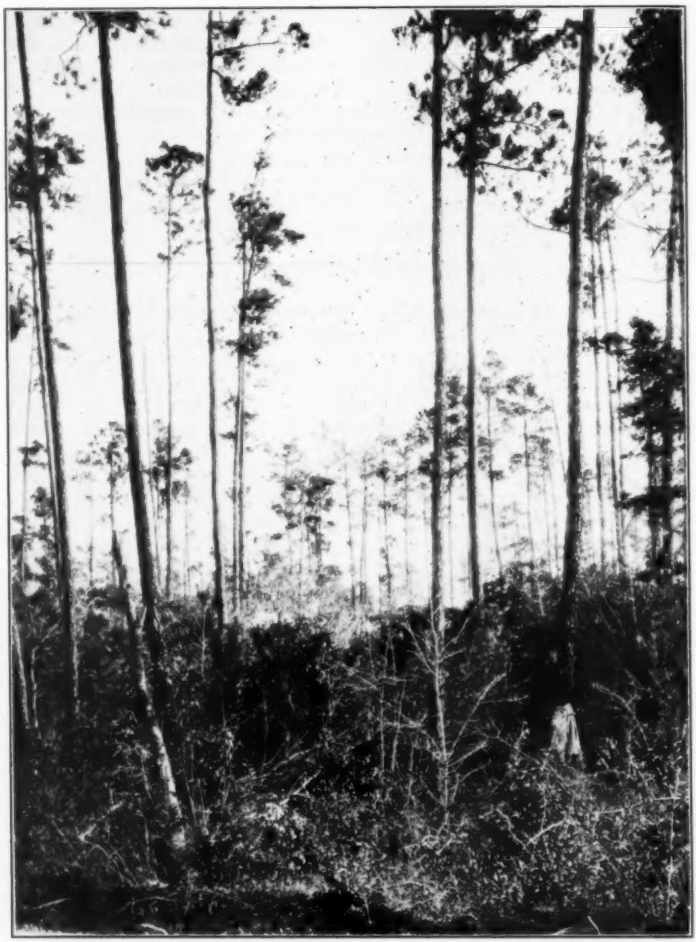


FIGURE 1.—Loblolly pine stand 11 years after it was cut to an elastic 10-inch diameter limit.

North Carolina. The stands had all been partially cut at least 10 years before and had since remained unburned. Figure 1 shows a typical stand. In general they were of the same age (45 to 60 years at the time

of cutting), had the same site index [5] (85 feet) according to determinations in near-by stands, had alike been cut to an elastic 10-inch diameter limit, and were approximately the same in density of stocking by basal area (50 to 70 percent of normal values (*13*) at time of cutting).

The diameter distributions in the several stands at the time of cutting were estimated from tree and stump tallies, on the basis of increment borings. The stand tables so reconstructed undoubtedly include errors due to the rough method of taking stand and stump tallies and to the lack of data on mortality of trees since the time of cutting. In general, however, they indicate a high degree of similarity

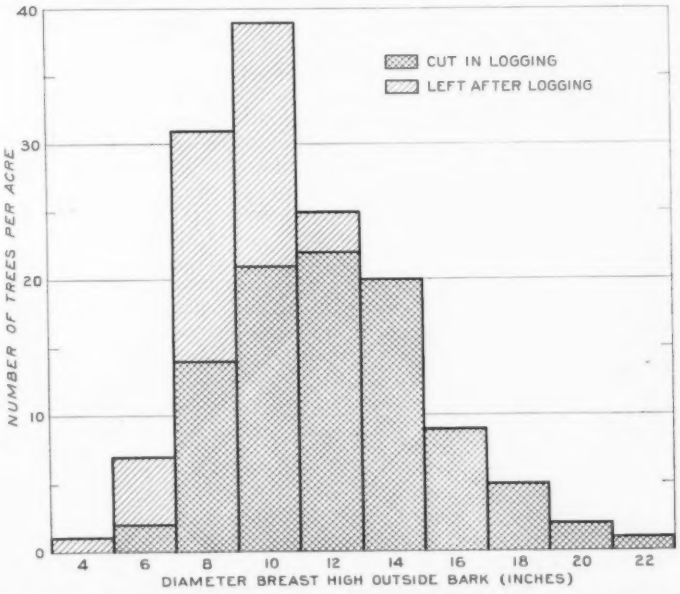


FIGURE 2.—Average distribution of trees cut and trees left in logging on study areas, by 2-inch diameter classes, according to reconstructed stand tallies.

among the nine stands as to density of stocking and as to representation of various diameter classes. Average stand conditions on the nine areas are presented graphically in figure 2.

A selected portion of each of the nine stands was examined in detail, the following data being taken for each of 1,464 trees:

Measurement	Method
Diameter at breast height	Diameter tape.
Total height	Abney level.
Height to base of crown	Do.
Crown width	Estimated to nearest foot.
Growth in stem radius at breast height for 5 years before and 10 years after release.	Measured from increment cores taken on south side of trees.
Total age	Increment cores.
Number and size of trees cut and trees left within 30 feet of the tree studied, and their position in relation to it.	Positions sketched on field sheet and diameters estimated in 2-inch classes.

[5] Average height attained by dominant and codominant trees at the age of 50 years.

ANALYSIS OF DATA

In order to analyze factors affecting increase in growth it was necessary to project many values back to the time of release. This operation involved material difficulties. In some cases it was facilitated by the use of detailed stem analyses of 84 sample trees selected at random.

A curve showing diameters inside bark corresponding to given diameters outside bark was set up from the values determined for these sample trees. The diameters inside bark of the 1,464 trees at the time of study were then read from this curve. When the diameters inside bark at different periods had been obtained for the individual sample trees by subtracting twice the radius increment as measured on the increment cores they were translated to diameters outside bark by reference to the same curve.

A curve relating height at time of release to height at time of study was likewise constructed on the basis of these stem analyses, and was used in estimating the heights of the study trees at the time of release.

A curve showing crown ratios [6] at the time of release corresponding to given crown ratios at the time of study was constructed from the stem-analysis data on the assumption that the height to base of crown of the individual trees had not changed since the time of release, and was used for projecting crown ratios of the study trees back to the time of release.

Estimation of crown width at the time of release was impracticable. Analysis of the growth of the branches on a few selected trees indicated that the horizontal expansion of the crowns was in most cases so small as to be negligible in comparison with the errors inherent in the methods of estimating crown width. Crown width estimated at the time of study was therefore used in the computations as crown width at the time of release.

The next step in the analysis of the data was to find a method of distinguishing, for individual trees, between (1) the growth that would have been made without release, and (2) the additional growth made, i.e., the growth attributable to release. The mean annual basal-area [7] growth at breast height for the 5-year period before release was taken as the equivalent of the growth that would have been made if the trees had not been released. This basis of comparison insured conservatism in estimates of growth increase due to release, since experience has shown that the rate of basal-area growth for individual trees in the lower crown classes, such as composed the residual stands included in this study, usually decreases and seldom or never increases unless the trees are influenced by changes in stand conditions. Accordingly, the portion of the growth attributable to release was computed for each tree as follows: (Basal area 10 years after release − basal area at time of release) − 2 (basal area at time of release − basal area 5 years before release). The values so obtained are herein referred to as increase in basal-area growth.

The increase in growth is graphically illustrated by the distance between the numerals 3 and 4 on the radius marked in figure 3.

The mean increase in basal-area growth shown by the study trees for the 10-year period following cutting was 0.093 ± 0.00185 [8] square

[6] Crown length in percentage of total height. All values considered in this paper are expressed in decimals.

[7] Cross-section area in square feet.

[8] Standard error $= \frac{\text{Standard deviation}}{\sqrt{\text{Number}}}$.

feet per tree, or 130 percent of the total mean basal-area growth which, according to estimates, the trees would have put on in that period if they had not been released. The smallness of the standard error of this mean in relation to the mean shows definitely that most loblolly pine trees left by cutting to an elastic 10-inch diameter limit in stands such as those studied increase in rate of basal-area growth during the 10 years following release. The chances are less than 1 in 1,000,000,000 that the mean basal-area growth of another sample of

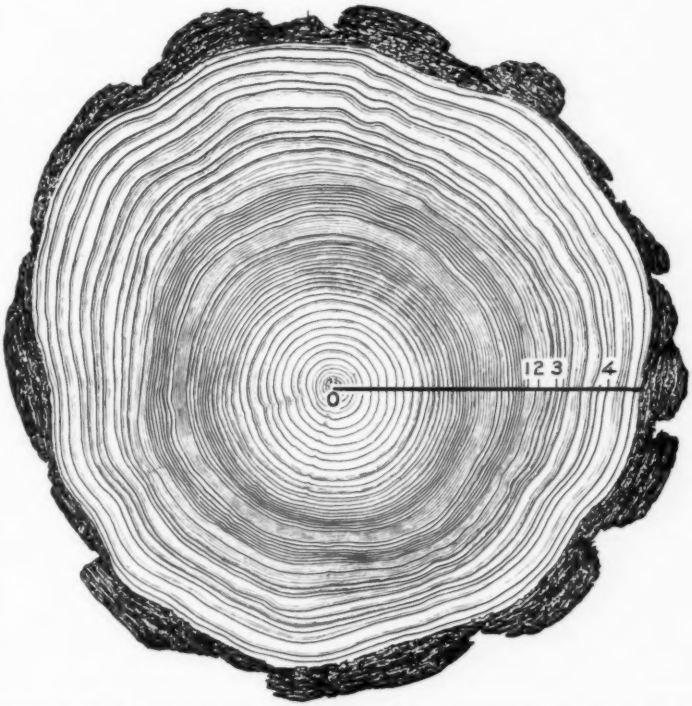


FIGURE 3.—Section of loblolly pine tree cut 12 years after release: 0–1, radius 5 years before release; 0–2 radius at time of release; 0–3, radius 10 years after release if basal-area growth had continued at the same rate prevailing in the 5-year period before release; 0–4, actual radius 10 years after release.

1,464 loblolly pine trees growing under like conditions (before and after release) would show no increase after release.

The change in rate of basal-area growth per tree in the 10 years following release varied widely, ranging from −0.10 to +0.45 square feet. A cursory examination of the data indicated that this wide variation was correlated in part with variations (1) in characteristics of the trees studied, hereafter referred to as tree factors, and (2) in degree of competition prior to release and degree of release from competition, which for the sake of brevity will be called the competition and release factors.

TREE FACTORS

The tree factors selected for consideration in the analysis were diameter, crown ratio, crown width, total height, and basal-area growth prior to cutting. In the sample studied the trees that had large diameters at the time of release showed less increase in basal-area growth than the smaller trees. The relationship between increase in basal-area growth and diameter was found to be distinctly curvilinear. Translation of diameter into basal area made the relationship linear (fig. 4, *A*).

Long-crowned trees plainly showed greater response to release than those with short crowns (fig. 4, *B*). Likewise, wide-crowned trees showed greater increase than those with narrow crowns (fig. 4, *C*). Trees about 60 feet tall showed greater increase in growth than either taller or shorter trees (fig. 4, *D*); this relationship, however, was not very distinct. No definite correlation was shown between basal-area growth during the 5 years before release and increase in basal-area growth after release (fig. 4, *E*).

It appeared that the factors just listed were intercorrelated and that the correlation of variations in any one of them with variations in increase in basal-area growth, the others being held constant, might be entirely different from the relationships shown in figure 4. Because the sample of trees was so small, it was impossible to subsort the data in such a way as to hold several factors constant. Mathematical correlation was chosen as the next best method of analysis, because it provides a measure of the relationship between two factors, others being held constant mathematically at their means.

Accordingly, increase in basal-area growth was correlated with the five tree factors considered in the foregoing, that is, basal area, crown ratio, crown width, and total height at time of release, and basal-area growth during the 5 years before release. The resulting equation was:

Increase in basal-area growth in the 10 years following release equals -0.1397 basal area $+0.2837$ crown ratio $+0.00163$ total height $+0.00633$ crown width -0.3196 basal-area growth during the 5 years prior to release -0.1116.

When the regression equation is interpreted in the usual way, that is, on the basis that the regression coefficient of any independent variable measures the change in the dependent variable associated with a unit change of that independent variable, the other independent variables being held constant at their means, it appears that the small, tall trees with long, wide crowns, which were growing the slowest before release, usually showed the greatest increase in basal-area growth after release. This conclusion was substantiated by the fact that all the regression coefficients were at least 4.5 times as large as their standard errors, proof that each factor was significantly correlated with increase in basal-area growth when the others were held constant.

When the regression coefficients were translated into β coefficients [9] and so made directly comparable, it was found that

β basal area $= -0.2906$
β crown ratio $= 0.3777$
β crown width $= 0.3736$
β total height $= 0.2408$
β basal-area growth during the 5 years before release $= -0.1151$

[9] β = Regression coefficient $\frac{\text{Standard deviation of independent variable}}{\text{Standard deviation of dependent variable}}$.

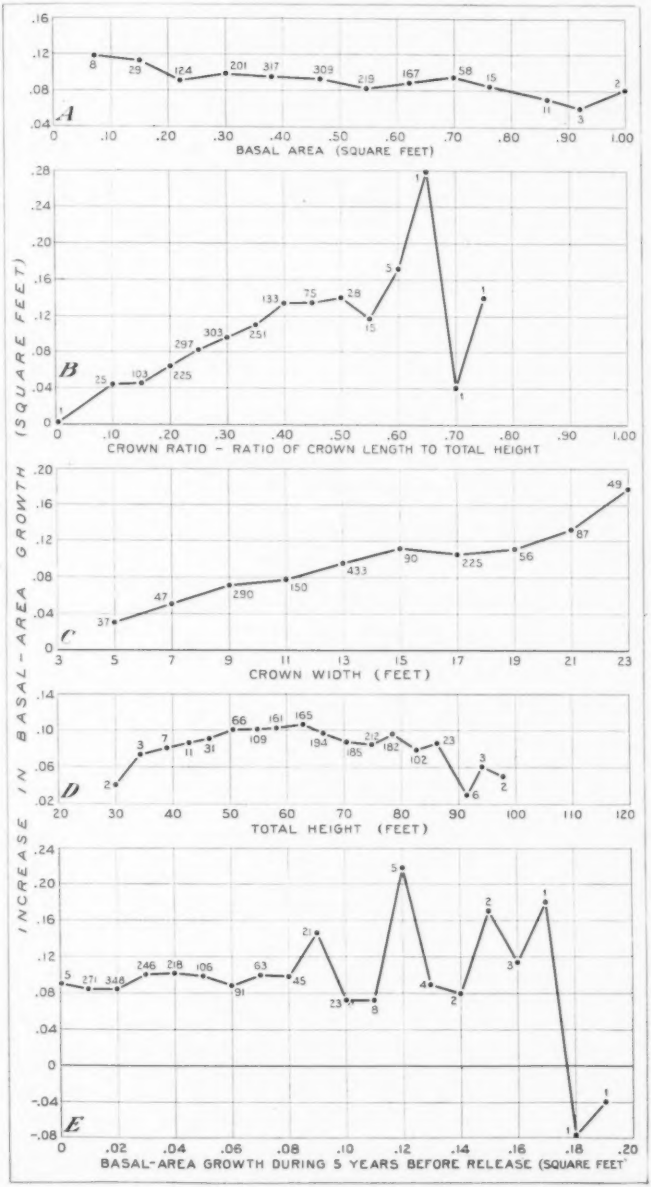


FIGURE 4.—Gross correlations between tree factors at time of release and change in rate of basal-area growth in the 10-year period following release. Numerals indicate number of trees in each class: *A*, Basal area (square feet); *B*, crown ratio (percentage of total height); *C*, crown width (feet); *D*, total height (feet); *E*, basal-area growth during the 5 years before release (square feet).

It is plain that, of the five factors considered, crown ratio and crown width had the greatest influence on increase in basal-area growth after release, and that basal-area growth before release had the least influence.

Although no significant gross correlation between total height and increase in basal-area growth was shown (fig. 4, *D*), a marked correlation between these factors appeared when basal area, crown ratio, crown width, and basal-area growth before release were held constant. Basal-area growth before release also showed much more definite correlation with increase in basal-area growth after release when the other tree factors included in the analysis were held constant.

COMPETITION AND RELEASE FACTORS

A consideration of increase in growth following release would not be complete without an attempt to measure the effects of various degrees of release on trees differing in characteristics and previously subjected to various conditions of competition. The first problem encountered in this phase of the work was the selection of measures of competition and release. Any measure or measures of competition should include number and size of competing trees; quantity of tree reproduction, of shrubs, and of herbs; the distance of trees and other competing vegetation from the tree studied; their position in regard to the tree studied and to each other; and their characteristics. No specific data were available on competing tree reproduction, shrubs, and herbs. Data on competing trees were available for all these items except the last from sketches taken for each study tree and its surroundings. It was impracticable, however, to consider in full detail even all of the factors for which data were available. Accordingly, the number and size of competing trees located between 0 and 10 feet, between 10 and 20 feet, and between 20 and 30 feet [10] of the tree studied were chosen as the factors best lending themselves to analysis of the small quantity of data at hand.

Several methods of evaluating these measures were tried and rejected because they were logically inadequate or plainly would not allow the use of the correlation method in the analysis. For example, total basal area of all the trees in each of the 10-foot zones at the time of partial cutting, and percentage of the basal area removed from each of the three zones, were rejected because when analyzed on this basis the data exhibited a definite tendency toward joint correlation.[11] Finally it was decided that analysis of competition and release should be based on the number of trees cut, the number of trees left, the average basal area of the trees cut, and the average basal area of the trees left, in each of the three zones.

A multiple correlation of increase in basal-area growth with these factors and the five tree factors previously considered, computed in an attempt to determine in what degree any one of the several factors was correlated with increase in basal-area growth, the others being held constant at their means, gave some results difficult to reconcile with past findings.

As is shown in table 1, the regression equation indicates that a variation of 1 in the number of trees left in the 10-foot zone was not

[10] These ranges of distance are hereafter termed the 10-, 20-, and 30-foot zones.

[11] Joint correlation is that type in which the degree of correlation between an independent variable and the dependent variable is governed by values of another independent variable.

associated with so much variation in increase in basal-area growth as a variation of 1 in the number of trees (of the same size) left in either the 20- or the 30-foot zone. This indication, which might be interpreted to mean that trees left in cutting suffer less competition from trees left within 10 feet of them than from the same number of trees separated from them by a distance of from 10 to 30 feet, is inconsistent with the general experience of woodsmen.

TABLE 1.—*Regression coefficients, and their errors, obtained in multiple correlation of certain variables with increase in basal-area growth of loblolly pine in the 10 years following release*

Variable[a]	b[b]	σ_b[c]	t[d]
Basal area of study tree at time of release	−0.1487	0.01530	9.72
Crown ratio of study tree at time of release	+.2729	.01877	14.54
Total height of study tree at time of release	+.0016	.00022	7.27
Crown width of study tree at time of release	+.0075	.00048	15.62
Number of trees left within 10 feet of study tree	−.0006	.00050	1.20
Number of trees cut within 10 feet of study tree	+.0159	.00252	6.31
Number of trees left 10 to 20 feet from study tree	−.0060	.01380	.43
Number of trees cut 10 to 20 feet from study tree	+.0032	.00102	3.14
Number of trees left 20 to 30 feet from study tree	−.0018	.00154	1.17
Number of trees cut 20 to 30 feet from study tree	+.0013	.00080	1.62
Average basal area of trees left within 10 feet of study tree	+.0067	.01015	.66
Average basal area of trees cut within 10 feet of study tree	−.0031	.00392	.79
Average basal area of trees left 10 to 20 feet from study tree	+.0108	.00593	1.82
Average basal area of trees cut 10 to 20 feet from study tree	+.0025	.00329	.76
Average basal area of trees left 20 to 30 feet from study tree	+.0108	.00587	1.84
Average basal area of trees cut 20 to 30 feet from study tree	−.0075	.00391	1.92
Basal-area growth of study tree in the 5 years before release	−.2238	07338	3.05

[a] Constant term is −0.1372.
[b] Regression coefficient denoting regression of variable on increase in basal-area growth, other variables being held constant at their means.
[c] Standard error of regression coefficient.
[d] Regression coefficient in terms of its standard error. Figures given in this column show by how many standard errors the regression coefficients are removed from 0. The limit of significance usually accepted is 2 standard errors.

Another relationship inconsistent with general observations is indicated by the coefficients of average basal area of trees cut and of trees left at different distances from the study trees. The coefficients as determined in the equation indicate that the removal of large trees from the 10- or 30-foot zones was associated with less increase in basal-area growth than the removal of large trees from the 20-foot zone.

These inconsistencies suggested that (1) important variables were not considered in the equation, (2) the relationships were curvilinear, (3) the relationships were joint, or (4) the relationships were joint and curvilinear.

Of these possibilities the first was not investigated, because data on other variables were not available. This lack of data was unfortunate, because it cannot be doubted that variations in several other tree factors, and in degree of competition from tree reproduction, shrubs, and herbs, had an important bearing on increase in growth.

The relationships were found to be linear, by the use of the Bruce and Reineke technic (*2*).

In investigating the third and fourth alternatives it was necessary to subsort the data according to different degrees of competition and of release from competition. The fineness of this subsorting was limited by the fact that so few data were available. Accordingly, the only competition and release factors recognized were number of trees

cut and number of trees left in the 10- and 20-foot zones. Even in these classes, the data in each group created by subsorting were very scanty. The largest general group, that with 0 trees cut and 0 trees

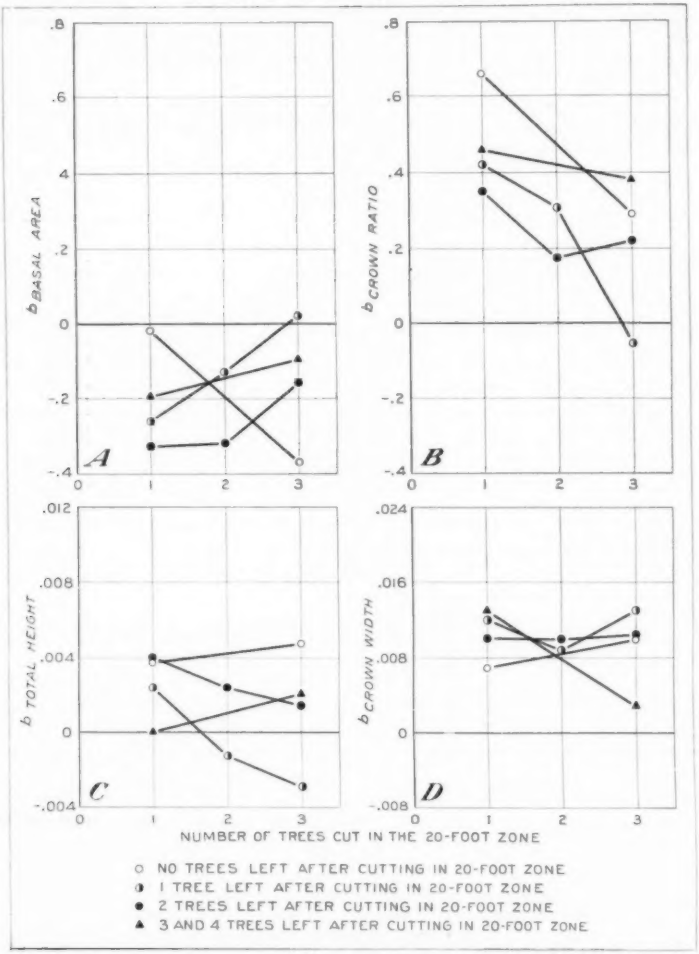

b BASAL AREA
b CROWN RATIO
b TOTAL HEIGHT
b CROWN WIDTH
A
B
C
D
NUMBER OF TREES CUT IN THE 20-FOOT ZONE
NO TREES LEFT AFTER CUTTING IN 20-FOOT ZONE
1 TREE LEFT AFTER CUTTING IN 20-FOOT ZONE
2 TREES LEFT AFTER CUTTING IN 20-FOOT ZONE
3 AND 4 TREES LEFT AFTER CUTTING IN 20-FOOT ZONE

FIGURE 5.—Relations between multiple regression coefficients of basal area *A*, Crown ratio *B*, total height *C*, and crown width *D* as correlated with increase in basal-area growth in the 10 years following release, and degree of release as measured by number of trees cut in the 20-foot zone when different numbers of trees were left following cutting. In all cases no trees were present in the 10-foot zone before or after cutting.

left in the 10-foot zone and with varying numbers of trees cut and left in the 20-foot zone, was chosen for the more detailed analysis. This group was subdivided into groups having constant numbers of trees cut and left in the 20-foot zone.

Table 2 shows the regression equations obtained by correlating increase in basal-area growth with four tree factors for each group.

Figure 5 indicates a possible correlation of the regression coefficients with degree of release as measured by number of trees cut in the 20-foot zone. For the study trees this possible correlation is most pronounced in the case of basal area and crown ratio; even in these cases it is questionable, because of the comparatively large errors of the regression coefficients, shown in table 2.

TABLE 2.—*Regression coefficients obtained by correlating increase in basal-area growth in the 10 years following release with basal area, crown ratio, total height, and crown width at time of release of study trees within 20 feet of which varying numbers of trees were cut and left, respectively, no trees being cut or left within the 10-foot zone*

Number of trees in 20-foot zone		Characteristics of study trees at time of release [a]								Number of instances
		Basal area		Crown ratio		Total height		Crown width		
Cut	Left	b [b]	σ_b [c]	b [b]	σ_b [c]	b [b]	σ_b [c]	b [b]	σ_b [c]	
1	0	−0.0153	0.1391	+0.6508	0.1914	+0.0038	0.0024	+0.0071	0.0046	22
2	0									
3	0	−.3747	.1388	+.2916	.1695	+.0046	.0022	+.0097	.0044	34
1	1	−.2584	.0954	+.4145	.1486	+.0024	.0014	+.0118	.0035	29
2	1	−.1322	.1160	+.3106	.1218	−.0012	.0015	+.0081	.0029	32
3	1	+.0159	.1223	−.0531	.1235	−.0025	.0019	+.0131	.0032	40
1	2	−.3283	.0952	+.3534	.1683	+.0039	.0025	+.0104	.0036	25
2	2	−.3243	.1238	+.1784	.1607	+.0024	.0022	+.0097	.0048	25
3	2	−.1606	.2397	+.2161	.2484	+.0014	.0033	+.0106	.0046	24
1	3 or 4	−.1978	.0916	+.4760	.1608	+.0004	.0016	+.0131	.0038	31
2	3 or 4									
3	3 or 4	−.0935	.0663	+.3887	.0908	+.0019	.0011	+.0029	.0022	30

[a] Independent variables.
[b] Regression coefficient denoting regression of independent variable on increase in basal-area growth, other independent variables being held constant at their means.
[c] Standard error of regression coefficient.

If these relationships are true, the degree of correlation between basal area at time of release and increase in basal-area growth after release tends to approach 0 as the degree of release is increased, competition before release and tree characteristics being constant. Likewise, such relationships might indicate that the degree of correlation between crown ratio and increase in basal-area growth approaches 0 as the degree of release is increased. No evidences of curvilinear relationships were found in these several equations.

PREDICTION MECHANISMS DEVELOPED, AND THEIR APPLICABILITY

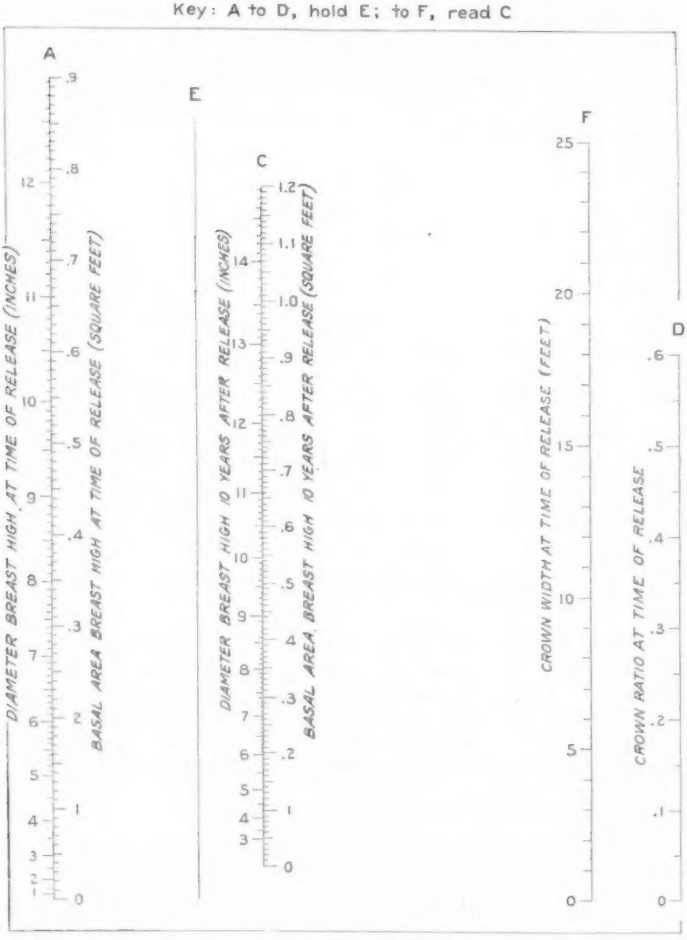
Analysis of the data available in this study has resulted in development of the following equations for predicting the sizes of individual loblolly pine trees 10 years after release:

(1) 0.8681 basal area (including bark) at time of release + 0.2914 crown ratio + 0.0065 crown width + 1.6332 basal-area growth during the 5 years before release + 0.0017 total height − 0.1212 = basal area (including bark) 10 years after release. Standard error of estimate = 0.0613 square foot.

(2) 1.0332 basal area (including bark) at time of release + 0.3116 crown ratio + 0.0088 crown width − 0.0602 = basal area (including

bark) 10 years after release. Standard error of estimate = 0.0726 square foot.

(3) 0.9564 basal area (including bark) at time of release + 2.2872 basal-area growth during the 5 years before release + 0.1015 = basal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A
E
C
F
D
DIAMETER BREAST HIGH AT TIME OF RELEASE (INCHES)
BASAL AREA BREAST HIGH AT TIME OF RELEASE (SQUARE FEET)
DIAMETER BREAST HIGH 10 YEARS AFTER RELEASE (INCHES)
BASAL AREA BREAST HIGH 10 YEARS AFTER RELEASE (SQUARE FEET)
CROWN WIDTH AT TIME OF RELEASE (FEET)
CROWN RATIO AT TIME OF RELEASE

FIGURE 6.—Alinement chart for predicting sizes of individual trees 10 years after release by partial cutting. The chart is based on the equation 1.0332 basal area (including bark) at time of release +0.3116 crown ratio +0.0088 crown width −0.0072 = basal area (including bark) 10 years after release.

area (including bark) 10 years after release. Standard error of estimate = 0.0708 square foot.

A consideration of the standard errors of estimate for these three equations shows that (1) is the most accurate as a predicting mechanism and that (3) is second in accuracy. For practical purposes equations (2) and (3) are more usable than equation (1), and they are therefore presented in alinement-chart form in figures 6 and 7.

The use of either of these predicting mechanisms requires caution. It must be remembered that they will give results within the standard errors presented only if they are applied to trees growing in stands

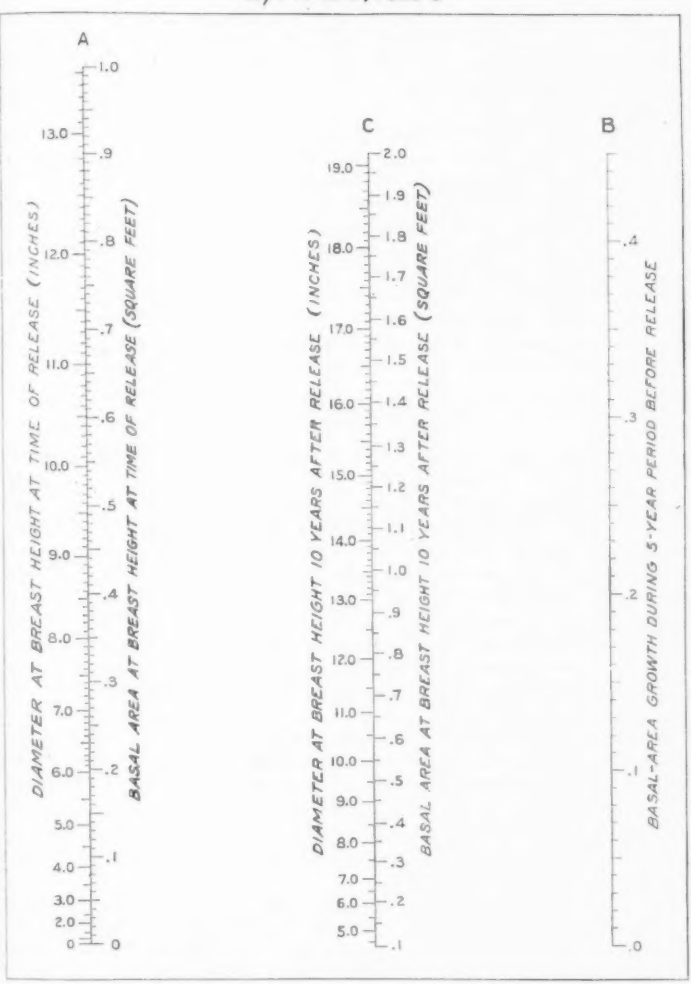
Key: A to B, read C
A
DIAMETER AT BREAST HEIGHT AT TIME OF RELEASE (INCHES)
BASAL AREA AT BREAST HEIGHT AT TIME OF RELEASE (SQUARE FEET)
C
DIAMETER AT BREAST HEIGHT 10 YEARS AFTER RELEASE (INCHES)
BASAL AREA AT BREAST HEIGHT 10 YEARS AFTER RELEASE (SQUARE FEET)
B
BASAL-AREA GROWTH DURING 5-YEAR PERIOD BEFORE RELEASE

FIGURE 7.—Alinement chart for predicting sizes of individual trees 10 years after release by partial cutting. The chart is based on the equation 0.9664 basal area (including bark) at time of release + 2.2472 basal-area growth during the 5 years before release + 0.1015 = basal area (including bark) 10 years after release.

similar to those included in this study and only if the stands have been or are to be cut to an elastic 10-inch diameter limit and the distribution by size classes of trees cut and trees left approximates that presented in figure 2.

It does not appear that these limitations will greatly restrict the use of the equations on stands cut over under present cutting prac-

tices. Most of the loblolly pine stands that are now being cut are between 40 and 65 years old. Likewise, most of them are between 50 and 70 percent stocked by basal area and are growing on areas with site indices between 80 and 90 feet. In present cutting practice the elastic 10-inch diameter limit is the prevailing standard. If the equations are to be applied under future conditions, however, it will probably be necessary to extend the study to include areas cut over to other diameter limits. The probability that revision will be needed to meet future conditions is particularly great as regards application of the equations to areas cut over for lumber, since recent studies (*6*, *7*) have shown that in lumber operations the removal of trees less than 12 inches in diameter breast high is in many cases unprofitable.

The apparent variations in the correlation of tree factors and growth increase on the one hand with different degrees of release on the other led to the conclusion that with the small quantity of data at hand the effects of different degrees of release on growth increase could not be satisfactorily analyzed.

From the regression equation including the five tree factors as independent variables the tentative conclusion may be drawn that in order to get the greatest basal-area increase per tree after release the small, tall trees with long, wide crowns should be left in cutting.

SUMMARY

The basal-area growth at breast height of loblolly pine trees released by cutting to an elastic 10-inch diameter limit was found to be 130 percent greater, on an average, in the 10 years following release than the growth which, according to estimates, the trees would have put on in that period had they not been released.

Variations in the growth increase of the individual trees in the stands studied were shown to be correlated in part with variations in individual tree characteristics. Crown ratio, crown width, basal area, and total height at the time of release, and basal-area growth during the 5-year period prior to release, were shown to be correlated with increase in basal-area growth after release, in degrees diminishing in the order named.

Measurement of the correlation between degree of competition prior to release and degree of release from competition on the one hand and increase in growth following release on the other was difficult because of the complexity of the relationships involved, including tree-factor relationships. No satisfactory method was found, in fact, for measuring the effects of different degrees of release on the growth of trees having the same characteristics.

Mechanisms were developed for predicting the sizes of individual trees 10 years after release. These are theoretically applicable only to trees growing under the same general conditions as the trees included in the sample, and to stands cut in approximately the same way as those sampled. Most of the loblolly pine stands now being cut in the Atlantic coastal plain are, in fact, highly similar in age, density of stocking, and size to the stands in which the study trees were growing prior to release.

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